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PRIMARY SPONTANEOUS SARCOMA IN MICE[1]

EIGHTH COMMUNICATION

MAUD SLYE, HARRIET F. HOLMES AND H. GIDEON WELLS

From the Otho S. A. Sprague Memorial Institute and the Department of Pathology of the University of Chicago

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The literature on tumors in mice does not furnish satisfactory information concerning the frequency of sarcomas among them. Statistics on other animals indicate that with most species, as with man, sarcoma is much less common than carcinoma. Caspar (1), in his review on the tumors of animals, published twenty years ago, makes the statement that sarcomas behave in animals quite the same as the corresponding tumors in man, and are more malignant than carcinomas, with much more extensive metastasis and rapid post-operative recurrence. They are, according to the literature reviewed by him, the most common tumors of dogs and horses, but it is to be considered that in the earlier literature there are many granulomas mistakenly diagnosed as sarcoma. Frohner (2), in 644 operated tumors in dogs, found 44 sarcomas. In rats, sarcomas seem to be especially frequent. McCoy (3) found, in 100,000 rats killed in plague work, 103 tumors, of which 30 were diagnosed as sarcomas and 18 fibromas. Of the sarcomas, 18 were found in the liver, 5 in the subcutaneous tissues, and 5 in the mesentery, and 1 each in the testicle and the pelvis. This predilection of the liver is corroborated by Woolley and Wherry (4) who found in 22 spontaneous tumors in rats, including 7 sarcomas, 3 sarcomas of the liver, which were, as in many of McCoy's cases, associated with parasites (5). In 250,000 ground squirrels McCoy

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[1] Presented before the American Association for Cancer Research, May 8, 1916.

(6) found 8 tumors, all in females. Of these tumors, 5 were diagnosed as sarcomas, there being no carcinomas found in these animals.

Wild animals in general would seem, as far as the very small number of recorded cases indicates, to have a relatively high incidence of sarcoma as contrasted with carcinoma. Of 34 tumors found in all sorts of animals in the Philadelphia Zoological Gardens by Fox (7), there were 18 epithelial tumors and 10 sarcomas. Of these 10 sarcomas, 4 were in birds, and, especially since Rous's studies, the relative frequency of connective tissue growths in birds is generally recognized. But even with the birds eliminated, the relative incidence of sarcoma is high in the wild animals. Of the 6 mammalian sarcomas in Fox's collection, 3 were in wolves, 1 in a male wild mouse (*Peromyscus leucopus*), 1 in a Dorcas goat and 1 in a zebra. The mouse sarcoma was spindle-cell, arising from the soft tissues of the right thigh, and metastases are not mentioned.

The most extensive data concerning spontaneous sarcoma in mice are furnished by Haaland (8). In 288 mice with 353 primary tumors (excluding lymphomatous growths, lung adenomas and sebaceous adenomas), there were 6 sarcomas and 339 epithelial growths. These figures do not give any indication of the actual frequency of sarcomas, as it is expressly stated that "These tumors were found in 288 mice at the time of first entering them in the laboratory register; tumors which developed later, or were only found at post-mortem examination, are not included in these figures (except in a few cases of rare tumors in the internal organs)."

Of Haaland's 6 sarcomas, 3 were typical spindle-cell growths, 1 arising in the pectoral muscles and 2 subcutaneously in the middle of the back; no metastases are described. In addition to these 3 there were: (4) a polymorphous-cell sarcoma which developed in the site of an old operation wound (this mouse also having a carcinoma of the ovary); there was a secondary sarcoma growth in the axilla. (5) An osteosarcoma the size of a "large walnut," in the thigh of a male mouse, recurring rapidly after partial removal but without metastases; this was inoculated into 800 other animals and round-cell sarcomas were obtained in 15. (6) An old female mouse, recovered from operative removal of a mammary carcinoma, developed a large-cell sarcoma of the spinal column, growing along the cord but not producing metastases. Also to be considered in this connection are the following: (7) A case described as carcinoma with sarcomatous formation, which on inoculation into other mice yielded only sarcomas; (8) a melanoma arising in the site of a slit in the ear of a black female mouse, fourteen months old, with possibly a secondary growth in the neck; inoculation unsuccessful in 121 mice.

The first six tumors were inoculated into 1364 mice, 846 of which lived over four weeks, and of these 68 or 8 per cent developed tumors. The tumors derived from the spindle-cell sarcomas were absorbed again after four to six weeks growth, but if transplanted before spontaneous absorption had begun they could be transplanted indefinitely. It will be noted that of the 6 sarcoma mice, 3 had other tumors, and 2 sarcomas arose definitely at the site of old injuries.

Murray (9) previously reported from the same institution, three cases of sarcoma, but does not indicate the number of mice from which the material was drawn, the number of other tumors in the stock, or other statistics that might be used for comparison. His tumors were: (1) A large spindle-cell intra-abdominal growth surrounding the right kidney, which could not be transplanted (sex of mouse not given); (2) Angiosarcoma or angioma in the left inguinal region of an adult male; inoculated into 82 mice without success. (3) A chondro-osteosarcoma 1.5 cm. in diameter, from the left groin of an adult female, removed by operation, with recurrence in three months infiltrating the peritoneum. This was successfully inoculated. In structure it resembled the mixed tumors common in the mammary gland of dogs. Haaland (10) has also described a chondro-fibrosarcoma arising from the vertebral column of an old mouse, sex not stated, invading the adjacent tissues but causing no metastasis.

Among Jensen's (11) early papers is described a mouse with spontaneous cancer which died and left four young; one of these developed an enormous intra-abdominal round-cell sarcoma. Further details are not given.

Jobling (12) described 41 tumors occurring in 26 mice, including 2 sarcomas. (1) Adult female, spindle-cell sarcoma arising below the left foreleg, infiltrating the chest wall and projecting into the pleural cavity; no metastasis. This mouse also had bilateral papillary cystadenomas of the ovaries. (2) Similar in location and structure to No. 1 even to the ovarian adenoma, with a primary lung tumor in addition. Forty mice were inoculated with fourteen takes, "now growing in the second generation."

Tyzzer (13) found one typical sarcoma among 83 primary spontaneous tumors in mice. "This tumor, like several of those already described, occurred in a family derived from a tumor parent." It was in a female, twenty-five months old, was located in the orbit and reached a size of 3 cm. in diameter, penetrating the skull and growing into a vein. From this tumor 11 mice were inoculated, but apparently the tumor was infected and all grafts sloughed out but one, which developed a transitory nodule. The primary growth was composed of atypical cells, mostly larger than connective tissue cells, many attaining the proportions of giant cells, and multinucleated cells were present. Fibroglia fibrils were also described. The lung showed minute metastatic growths. He also described a mouse with many small nodules in the liver, and similar tissue in the spleen, resembling fibrosarcoma, also three other cases with peculiar cellular growths in the liver and spleen that were not classified.

Apolant (14) described a sarcoma arising from the femur in a male mouse, this being the only male with spontaneous tumor in a series of 800 cases. The growth was an osteoid sarcoma arising in the periosteum of the left femur, exhibiting varying structure in different parts, some areas being composed chiefly of spindle cells, while others resembled carcinoma sarcomatodes. Inoculated into 100 mice six takes were obtained, with 100 per cent positive results in later generations, the growth becoming more and more like a round-cell sarcoma.

In the limited material collected above from the literature, which does not include the cases of sarcoma arising from transplantation of supposedly pure carcinoma, we note the following facts: (1) in proportion to the cases of carcinoma found in mice, sarcoma is very infrequent—much more so than seems to be the case in other animals. Of course the readiness with which mammary gland cancer is recognized elevates that type of tumor into an improper prominence, for our own figures show that when systematic autopsies are performed on mice dying a natural death, other tumors are more frequent than current statistics indicate. (2) As the diagnosis of sarcoma is always the least certain of any differentiation in tumor pathology, and especially so when the numerous peculiar types of granulomatous growths of lower animals are taken into consideration, it is apparent that proper conservatism has been used in animal cancer work, or the proportion of growths called sarcoma would be higher. (3) Of the 17 cases collected, in 2, the sarcoma was definitely recognized as arising at the site of an old trauma, and in 5, other tumors were described in the sarcoma mouse. (4) Sarcomas of mice can be transplanted successfully into other mice, but apparently not more readily than carcinomas, indicating the improbability of infective granulomas having been mistaken for sarcomas. (5) Even in so small a series of cases, an extensive variety of histological structure is seen, spindle-cell sarcomas predominating. (6) Some of the sarcoma mice were males, although as the sex was not stated in all the cases reported, the proportions cannot be determined. (7) In only two of these cases were metastases recorded, indicating that in sarcomas as well as in carcinomas of mice, metastasis is not so frequent as in human cancer. (8) In a few cases the sarcomas were recorded as occurring in "cancer families."

INCIDENCE OF SARCOMA IN THE SLYE STOCK

In 12,000 mice dying in this laboratory, and subjected to careful autopsy, we have found 87 with neoplasms that can be diagnosed as nothing except sarcoma, an incidence of about 7.35 per thousand, including animals of all ages and dying from whatever cause, whether accidental or natural. We recognize fully the difficulties that attend the differentiation of sarcoma, and for the purpose of this study have excluded every form of new growth concerning the nature of which there seemed any possible room for question. Therefore, we have not included numerous cases in which we think that the growths are probably sarcomatous, and many more in which we cannot be sure that the neoplasm is not sarcoma. The many lymphomatous neoplasms are also omitted, under which term we include not only those conditions which resemble human leukemia and pseudoleukemia, but also many other growths of lymphoid character with marked local infiltrative character but lacking the tendency to generalization exhibited by the foregoing; this latter type of growth corresponds very closely, often exactly, to what are designated as lymphosarcomas in man, and have been observed by all workers with mouse tumors. We also have omitted a series of neoplasms arising in the testicle, ovary and adrenal, which, as with tumors arising from the same tissues in man, often resemble sarcomas closely; for these we prefer Adami's designation of "mesothelioma" and plan to consider them more extensively in a later paper, for they constitute an interesting group.

Having thus omitted every debatable form of neoplasm, and having also excluded every growth that gave any indication of a possible inflammatory origin, we feel assured that these 87 neoplasms are all true sarcomas as judged by the most rigid standards. We may add that numerous borderline cases have been kindly studied by Dr. E. R. Le Count, and in each instance when any one of the examiners was in any way uncertain as to the sarcomatous nature of the neoplasm it has not been included in this series.

On the other hand, the statistical value of our figures is lessened by the fact that we have undoubtedly omitted some growths that are true sarcomas. Our figures represent minimal values only, and especially so as to lymphosarcomas which are excluded entirely. A special study of the lymphomatous and leukemic conditions of mice is now under way, and it is hoped that when this is completed we shall be able to speak with more assurance as to the identity of growths of this class.

From the standpoint of investigations in heredity, with which our work is particularly concerned, it is just as undesirable to call a sarcoma something else as to include a granuloma among the sarcomas, and hence the rigid classification adopted in this study of sarcomas is no more satisfactory for our heredity statistics than would be a lax classification that included some growths of doubtful nature. Therefore, in charting the heredity statistics it is necessary to recognize the absence of positive criteria for the differentiation of sarcoma, and to admit the borderline cases with a mark of interrogation to indicate this fallibility.

We would also emphasize the character of the material from which these sarcomas have been obtained, and the conditions under which the growths have developed. The 12,000 mice are all the descendants of a limited and carefully selected stock, bred together according to definite plans designed to give evidence as to the influence of heredity upon the incidence of spontaneous tumors in mice, and, hence, including strains of highly cancerous ancestry and strains with ancestry free from cancer. They represent strains in which cancer is very common, strains in which it practically never occurs, and strains of intermediate character. The influence of heredity on the incidence of sarcoma will be considered elsewhere, and we mention these facts here to indicate the character of the material in this respect. It must also be emphasized that none of these mice has been subjected to any artificial influences that might modify its life. In no case is a spontaneous tumor used for inoculation, or operated upon, and no mouse born in this laboratory is ever used for any experimental work whatever. From the moment of its birth every effort is directed to the one object of permitting each mouse to reach a maximum age. Long experience and great care have made it possible to limit to a large extent the epidemic infections that constantly threaten such large colonies of mice under even the best of conditions. Of especial importance is the fact that every mouse that dies is submitted to a careful post-mortem examination, no matter whether it dies in infancy, from an accident, or from any other obvious cause; and every suspicious area is submitted to microscopic examination by three people or more. Were it not that every dead mouse is thus thoroughly investigated, and that the average age at death is, for a mouse community, very high, we should not have nearly so much tumor material to describe.

Of the 87 sarcoma cases, 30 were males and 57 females, a ratio of almost exactly 1 to 2. These figures may be compared with the statistics of the other tumors of the Slye stock that we have studied in detail; namely, the tumors of the lung and the liver. In the lung tumors (160 cases) there were 42.6 per cent of males and 57.4 per cent of females; in the liver tumors (28 cases) there were equal numbers of each sex. It is evident that these figures fail to bear out the prevalent statements concerning the great infrequency of tumors in male mice. The explanation of the discrepancy undoubtedly lies in the fact that most of the figures in the literature are derived from material selected by external examination, and from breeding stocks with a preponderance of females, so that the common mammary gland tumors constitute by far the greater part of the published material. Our material, coming from mice living out their complete span of life in as favorable conditions as possible, with more nearly normal proportion of sexes, and every death followed by thorough post-mortem examination, gives an accurate picture of the natural proportions of malignancy in the two sexes.

The reason that the females outnumber the males so much much more in the sarcoma series than in either the liver or the lung tumors, is probably that a large proportion of the sarcomas arise in the mammary gland. As we have not yet worked over the entire tumor material from these 12,000 autopsies we cannot state the exact proportion of sarcoma to carcinoma in the mammary gland.

DISTRIBUTION OF SARCOMA

By far the commonest point of origin of these tumors is beneath the skin, including both those that develop from the subcutaneous tissue proper and those that arise from the mammary gland. Forty-five, or almost exactly one-half, had this origin. Because of the widespread distribution of the mammary gland, it is usually impossible to tell with certainty whether a subcutaneous tumor on the trunk of a mouse arises from the stroma of the mammary gland, or arises from the subcutaneous tissue or muscle fascia and infiltrates the gland tissue. There are certain tumors that arise and develop in such a manner as to indicate strongly that their point of origin is in the mammary gland. When first observed, having then usually the size of a green pea, their location is the same as the carcinomas of the mammary gland, for which they are easily mistaken; like the carcinomas they appear especially in the anterior and posterior pairs of mammae, and a large percentage of those in mated females become apparent at the time of the birth of a litter. With some of them the histological evidence also supports the view that they arise in the mammary gland, because they are largely limited to the mammary gland substance. Often the relation to the tubules is close, resembling in a few instances a pericanalicular fibrosarcoma (fig. 1).

Taking into account the clinical history, the autopsy findings, and the histological evidence of these 45 superficial tumors, 13 seemed certainly to arise in the mammary gland, 10 others probably had the same origin, while with the remaining 22 there was either a lack of satisfactory evidence for this origin, or, in some cases, convincing evidence that the mammary gland was not concerned.

No other one location yielded many sarcomas, but next in order was the osseus and periosteal tissue, with eleven, distributed as follows: Jaw, 1; femur, 4; ribs, 3; foreleg, 2; knee, 1. Several other tumors were attached to bone, but apparently had arisen elsewhere, although in two or three cases this could not be positively determined, as, for example, tumors arising in the orbit.

Other locations which seemed to be the starting point of the sarcomas, as well as could be determined, were: Mesentery, 3;

head, 1; testicle, 1;[2] tail, 1; retroperitoneal, 2; neck, 1; pelvis, 1; ovary, 1; omentum, 1; orbit, 3; abdominal wall, 2; mouth, 1; arm, 1; thigh, 2; scrotum, 2; kidney, 2; uterus, 2; liver, 2; chest wall, 1.

In a few of these instances the tumor had involved several structures so extensively that the point of origin could not be stated exactly, and is only approximated; e.g., neck, pelvis, head, thigh, arm, orbit, chest wall.

UTERINE SARCOMA

The relative infrequency of uterine tumors in all mammals except man is, together with the similar peculiar frequency of human gastric carcinoma, one of the striking features revealed by comparative cancer studies. We have never found a carcinoma that arose primarily in the uterus, nor a leiomyomatous tumor, although Haaland (15) described a typical uterine fibromyoma. In this series, there are, however, two cases of sarcoma of the uterus.

10042. An old female mouse, with senile atrophy of most of the organs, exhibited a bilobed pelvic mass, occupying the site of the uterus. The right lobe measures 20 by 14 by 12 mm., and is pink and of fleshy consistency; the left lobe is paler in color, and measures 18 by 14 by 12 mm. The cut surface yields no exudate. The mass binds together the neck of the uterus, the urinary bladder, and the ureters, but does not involve the vulva (fig. 2). There are no metastases, and the ovaries are not involved. Microscopically all parts of the growth are alike, consisting of a typical spindle-cell sarcoma, with cells densely packed together and but little fibrillar material. In some places the tumor infiltrates the uterine muscular coat and involves the serosa; in others it compresses the muscular wall, as if growing out from the submucosa. Mitotic figures are not numerous.

10828. The right horn of the uterus is enlarged to form a soft fleshy mass, 12 by 10 by 10 mm., which contains no pus or products of conception. The left side of the uterus is not involved, and both ovaries seem to be normal. On the diaphragm is a nodule, 2 mm. in diameter, of tissue similar to the horn of the uterus. The regional lymph glands are somewhat enlarged. Death apparently resulted from a suppurative pyelonephritis.

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[2] This was a true spindle-cell sarcoma arising at the site of a wound, and entirely different from the more common "mesothelioma" of the testicle.

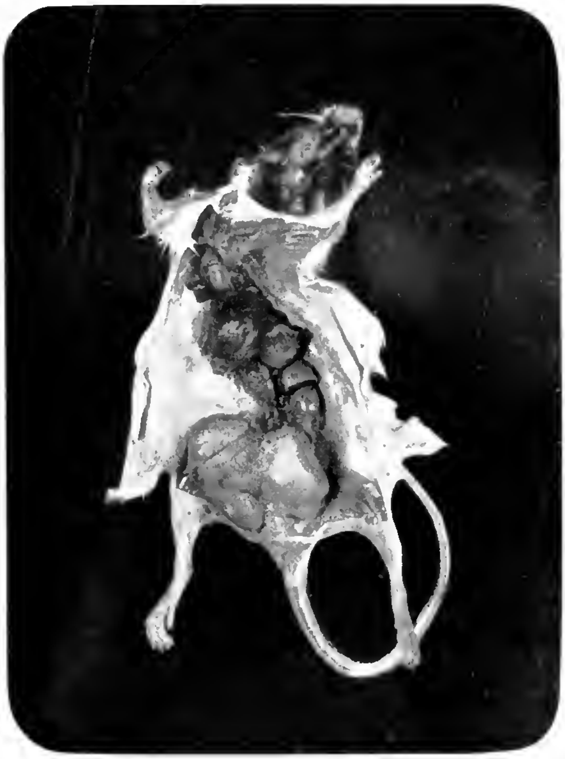

Fig. 2. Sarcoma of the uterus, involving both horns. Part of left horn has been removed for microscopy. Structure large spindle-cell sarcoma.

Microscopically the growth is composed of large spindle cells infiltrating the wall of the uterus in all parts; where under pressure the cells are somewhat smaller. Mitoses are scanty. The diaphragmatic nodule is composed of typical large spindle-cell sarcoma tissue, infiltrating the muscle freely, and with many mitotic figures. The regional lymph glands are hyperemic and hyperplastic, but show no tumor tissue.

It is an interesting fact that in 12,000 autopsies with approximately 2000 tumors there have been but two uterine tumors, both sarcomas, and that these two animals were closely related, 10,042 being the half aunt of 11,828 through the male line. The strain from which they come carries a very high incidence of cancer.

MULTIPLE PRIMARY SARCOMA

There were a few instances in which there seemed to be more than one primary sarcoma in the same mouse. These were as follows:

3117. A male mouse bitten on the back and on the genitals. From each of these sites arose a large spindle-cell sarcoma, alike in structure in each case, but developing synchronously so that there seemed little probability of either being secondary to the other. The genital tumor seemed to arise from the testicle, and was a typical spindle-cell sarcoma, apparently not of the more usual mesothelial type of testicle tumors. There were no metastases.

3444. Female. Here a spindle-cell sarcoma arose on each side of the chest, one being of a larger type of cell than the other. There was a pleural metastasis from the larger cell growth. The difference in structure and clinical course indicated, but did not positively prove them to be independent primary growths.

6629. A female mouse had a large tumor develop on each side of the face. As they arose independently it was thought during the life of the animal that the growths were both primary. At autopsy there was not sufficient difference in histology of the growths to establish this.

✓ 9741. Also a doubtful case. A female mouse showed a primary nodule in the ribs, and very soon thereafter a growth in the knee which grew more slowly. Both were osteosarcomas, so it is impossible to be sure whether the knee growth was secondary to or independent of the former.

8289 A female mouse had two perfectly distinct tumors, one arising near the spinal column and extending towards the left axilla, the other arising in the right chest wall. As these are both spindle-cell sarcomas of similar structure their true independence is not established, although probable.

It will be noted that in all these cases the structure of both tumors in each mouse was nearly or quite identical, so that only gross anatomical and clinical reasons exist for considering them as primary multiple tumors. In the first case the evidence of independence is better than in any of the others. In compiling our statistics each of these cases has been counted as one only, and not as two instances of sarcoma.

CO-EXISTENCE OF OTHER TUMORS

In several instances the mice with sarcomas also had elsewhere in the body a tumor of some other type. This tendency to multiple tumor formation has been noted by all investigators of spontaneous tumors in mice. There were in all 20 such cases, or about 23 per cent of the sarcoma mice. In our series of 160 lung tumors, we noted 23 primary tumors in other organs, or 14 per cent; in the liver tumor series, in 28 mice 7 had tumors in other organs, or 25 per cent. Apparently, then, sarcomas show about the same frequency of co-existence with other tumors as do the hepatic and lung tumors.

Of the 18 co-existing tumors, the lung tumors are decidedly the most common, there having been 11, 5 of malignant character and 6 benign. (The characteristics of these lung tumors are discussed in the fourth paper of this series.) There were 4 mammary gland carcinomas and one tumor of the mammary gland that seemed to be a true benign adenoma. The other tumors were liver adenoma, a squamous-cell carcinoma of the mouth, a perivascular "mesothelioma" of the testicle, and an ovarian adenoma. Not included are two cases of leukemia; there were no cases of pseudoleukemia accompanying typical sarcoma.

In two cases we have what seem to be co-existent sarcoma and carcinoma arising in the same or adjacent tissue, and constituting independent double tumors rather than mixed tumors or carcinoma sarcomatodes.

8560. Spindle cell sarcoma of jaw with squamous-cell carcinoma arising in the overlying mucous membrane. This was an old female mouse of a strain (146) characterized by high cancer incidence. There arose a lump in the lower jaw, the surface of which eventually became ulcerated, but the duration of the tumor was much longer than is usually the case in squamous-cell carcinoma. Microscopically the growth consists of two definite structures (fig. 3). The greater part is composed of a growth resembling typical spindle-cell sarcoma, with many large atypical multinuclear and uninuclear giant cells. Apparently this part of the tumor arose in the periosteum of the mandible, and shows more of the giant-cell sarcoma character than any of the other tumors that we have observed. It infiltrates the muscle and erodes the bones freely. Infiltrating this spindle cell growth from the surface is a typical squamous-cell carcinoma with marked hornification; it seems to have arisen from the mouth epithelium and not the skin. This epithelial growth in places extends quite deeply into the sarcoma-like tissue, and it too erodes and infiltrates bone tissue. There seems to be a slight inflammatory reaction about the epithelial neoplasm within the spindle-cell tissue. Mitotic figures are common in the carcinomatous tissue and much less abundant in the spindle cells. There were no metastatic growths found, but in the lung was a small primary adenomatous nodule. There is no sharp line of demarcation between the two types of growth, and in places there are features suggesting a transition of one into the other. Dr. James Ewing, who has kindly examined this tumor, believes it to be all carcinomatous, and a dentigerous origin is certainly possible. The resemblance to sarcoma is, however, too striking to be entirely dismissed. This growth is not included among our sarcomas, because of the doubt as to its origin.

8889. Spindle-cell sarcoma of the mammary gland adjacent to a carcinomatous growth. A female mouse, with a tumor arising in the left axilla, extending down the entire left arm to the hand, and across the chest to the right axilla. The entire tumor mass measured 50 by 40 by 40 mm., and seemed to consist of three coalescent nodules, one hemorrhagic, one largely necrotic, and a third more fleshy tissue without necrosis. In the left flank is another nodule 6 mm. in diameter, which seems to be a cystic lymph gland without neoplastic involvement. The lower lobe of the left lung is riddled with minute tumor nodules, but there are no metastases elsewhere. The large tumor of the mammary gland is found to consist of two parts; one, the larger, is a typical hemorrhagic cystic carcinoma with no noticeable peculiarities. This corresponds to the hemorrhagic part of the tumor as described above. Adjacent to this is a tissue composed solely of spindle cells, with but little formation of fibrillar substance, which evidently constitutes the greater part of the tumor. These cells grow up to the connective tissue surrounding the carcinoma, but do not invade it although not sharply walled off; there is none of this tissue found within the carcinoma itself. Towards the center of the spindle-cell growth the cells are less numerous and there are various degrees of degeneration and fibrosis. Mitotic division seems to be less abundant than amitotic in this part of the tumor. The sarcoma invades the adjacent muscle freely, but the regional lymph glands are not affected. In the lungs are many metastatic nodules which exhibit only the carcinomatous elements.

These two tumors have no intimate connection with one another, and we find no evidence to indicate that one bore any etiological relation to the other. We can merely say that we have a carcinoma and a sarcoma arising side by side in the mammary gland; consideration of their relation would be only speculation. This case somewhat resembles the one reported by Haaland (16).

TYPES OF SARCOMAS REPRESENTED

Spindle-cell sarcomas were by far the most abundant, constituting 47, or a little over one-half of the tumors. These illustrated all possible types, from the fibrosarcomatous, or "desmoid" growths (fig. 4), to the very large cell sarcomas with uninuclear and small multinuclear giant cells (fig. 5). It seems unnecessary to describe the characters of these tumors in detail, for they correspond in structure absolutely with the sarcomas occurring in man and given the same designations. Ten were diagnosed as round-cell sarcomas, ten were called "polymorphous"-cell sarcoma, and three seemed best described as oval-cell. Among the "polymorphous"-cell sarcomas were three or four which contained so many extremely large uninuclear cells that they might be entitled to the designation of giant-cell sarcoma. There were no sarcomas showing typically the myeloid type of giant cells, nor have we observed multiple myelomas or melanotic sarcomas; it may be recalled that Haaland reported a melanoma arising in the ear of a mouse.

A few of the sarcomas show a perivascular arrangement that resembles the structure commonly diagnosed as a perithelial sarcoma or hemangiosarcoma; three tumors showed this character conspicuously (fig. 6), arising one each from the scrotum, mammary gland and omentum. Two sarcomas only seemed to be entitled to be called alveolar sarcomas, and these were not very typical examples of this type of growth.

Twelve of the growths were osteosarcomas or chondro-osteosarcomas, and these presented many interesting features. They were usually very large and hard, and produced more extensive metastasis than any other type of neoplasm that we have found in mice. Because of their peculiarities they seem worthy of special consideration.

They occurred equally in both sexes, six in each. Only three failed to show metastases recognizable with the naked eye, an incidence of 75 per cent, which is, as far as we have read or observed, unequalled in any other type of neoplasm in mice if we exclude the lymphomatous new growths as of doubtful character. The distribution of the metastases was extensive—they occurred six times in the lung, five in the liver, twice each in mediastinum and diaphragm, once each in spleen, lymph glands, and knee (the last possibly being an independent primary tumor). Five arose from the hind leg, apparently the femur, with pathological fracture in three; four from the subcutaneous tissue or mammary gland, and three appeared to start from the chest wall or ribs. In general, they correspond closely to the case described by Murray, in which the tumor arose in the mammary region, and, as he remarks, they resemble the osteoid tumors found not infrequently in the mammary gland of the dog. Haaland, as before mentioned, also observed a similar tumor arising in the thigh of one of his mice, and in the spinal column of another.

An idea of the extent of these osteosarcomas and their metastases may be obtained from the following abstracts of the autopsy records of two cases:

9554. Female. Left femur spontaneously fractured near the hip, February 25, 1915; when the animal died on March 1 the left hind foot was necrotic. A tumor mass measuring 20 by 16 by 16 mm. arose apparently at the hip and spread up into the pelvis and retroperitoneal tissues; color, white; consistency, hard. It had displaced the left ureter which lay ventral to the tumor and passed diagonally across it. It also involved the peritoneum and the body wall; in the thigh it extended nearly to the left knee. The urinary bladder was distended and the left kidney cystic. The right kidney was buried beneath the right lobe of the liver, which was adherent to the intestines and to the parietal peritoneum. The liver was about twice the normal size, riddled with secondary tumor nodules from the pinpoint to 3 mm. in diameter. There was also an abscess about a tapeworm which extended into the common bile duct and intestines. The spleen was enlarged and contained in its upper surface a tumor nodule 1 mm. in diameter. Both lungs were riddled in all parts by secondary tumor nodules.

7983. Male. At the base of the ribs on the left side, and involving also the adjacent abdominal wall, was an extremely hard mass of tissue about 8 mm. in diameter, nearly spherical, white, with blood vessels passing over its surface. Adjacent thereto and further dorsal was a second mass of about the same size, somewhat less hard, reaching almost to the dorsal midline. Attached to the upper surface of the diaphragm was a similar, extremely hard mass, about 2 mm. in diameter. The upper part of the right lung was riddled with small hard tumor nodules, with a few softer nodules in the lower lobe. There were also tumor nodules in the posterior mediastinal tissues about the hilum of the lungs.

The histology of these tumors varies considerably. In some the primary growth and metastases are all composed of typical osteoid tissue, with trabeculæ of hyaline osteoid tissue showing greater or less calcium deposition in the centers and more cellular peripheries, often with fat tissue between the trabeculæ. Some show much calcification, some little, and true cartilage and bone are always scanty. In most instances the character varies considerably in different parts, there being areas of spindle or polymorphous cell sarcoma tissue with little or no osteoid tissue; necrosis, hemorrhage and mucoid degeneration are common. The metastases also vary in structure, but usually show a more pure type of osteoid sarcoma with a radiating structure and lobulated periphery, forming a striking picture (fig. 7).

In two cases, the livers of these mice showed most remarkable changes, consisting of isolated islands of fatty tissue, in which no liver cells remained in recognizable form, even as fatty metamorphosed liver cells (fig. 8). At first glance the impression is obtained that these are islands of simple fatty infiltration of the liver, but it is soon noted that there are generally more nuclei present than is usual in such a condition, and that these are not the ordinary flattened liver cell nucleus of the signet ring type of cell. Furthermore, the mouse liver is not much inclined to fatty infiltration of the type common in the liver of man and many other animals. In some islands it is at once apparent that this fatty tissue is replacing tumor nodules in the liver. In these earlier stages the number of round cells is so great that, admixed with the fat spaces and capillaries, the picture is that of fatty marrow associated with the osteoid tissue of the metastatic growth (fig. 9). The course of events in these two cases seems best interpreted as follows: The earliest stage of the metastatic growths is simply that of an osteoid sarcoma, about which there soon is laid down a collection of lymphoid cells resembling those of the marrow. In this cellular tissue fatty areolar tissue appears, increasing in amount with atrophy of the tumor tissue until all that is left is an island of fatty tissue resembling fatty marrow. Later the lymphoid elements disappear still further, leaving merely a well defined area of fatty areolar tissue surrounded by practically intact liver tissue.

Such a process we have never seen described in connection with tumors, and it would be less comprehensible if we did not know that any tissue that becomes calcified is likely to subsequently develop not only true bone tissue but also true marrow tissue, evidently carrying on the functions of marrow. By this process we may come to have marrow tissue in calcified heart valves, sclerosed vessels, calcified contents of the eye, and healed pulmonary lesions. The difficulty of this explanation for our liver nodules lies in the complete disappearance or absence from some of the fatty areas of all tumor tissue, and the fact that the fatty marrow-like tissue surrounds the osteoid tissue instead of being enclosed by it as an ordinary pathological marrow formation. However, we have been unable to find a better explanation which agrees with the fact that this hepatic lesion has been found only in these two cases of osteosarcoma, and that the fatty areas are associated with metastatic growths in many instances.

MIXED TUMORS

Besides the two instances described above in which carcinoma and sarcoma seemed to arise side by side, but apparently independently, we have three cases in which the structure of the tumor suggests a combination of epithelial and sarcomatous elements. These are not included in our list of 87 sarcomas.

3383. A female mouse with two areas of tumor growth in the mammary gland. Death from amyloidosis and hypertrophy of the heart. One tumor on the left side slightly ulcerated, measuring 22 by 15 by 9 mm., was a tubular carcinoma, without anything out of the ordinary about its structure. The other, 12 mm. in diameter, also ulcerated, in the right inguinal region, consisted of two distinct elements and its structure varied in different parts. In some areas the growth resembled in most respects a tubular carcinoma, with small cystic spaces lined with low cuboidal or flattened epithelium; but even in these areas most resembling carcinoma there are accumulations of spindle cells between and surrounding the tubular structures. These spindle cells are of medium size, with a nearly solid, deep staining nucleus, and but little discernible cytoplasm. In the greater part of the tumor these spindle cells are the predominant element, forming accumulations of a partially concentric structure and separated by a loose connective tissue which is not well defined. Even in the latter areas, tubular structures occasionally appear. The character of the cellular elements, and especially their relation to each other, recall the embryonal adenosarcoma of the kidney, which this tumor resembles closely in microscopic features, a resemblance observed by all the pathologists who have examined it. However, in this mouse the kidneys were free from tumor growth, and there were no metastases of the mammary gland tumors. Furthermore, we have never seen an embryonal adenosarcoma of the kidney in mice, and know of no such tumor having been reported in the literature. In favor of the sarcomatous, or at least the mesodermal origin of this tumor, is the presence, in both the stroma and some of the tumor nodules, of a type of mucoid or myxomatous degeneration similar to that found in connective tissues. This tumor agrees in many respects with the standards set for endotheliomas, which Hansemann maintains is the proper designation for most of the mammary gland tumors of mice.

3413. A female mouse (related to 3383 in that its aunt, 529, was a second cousin of 3413), died of acute pulmonary infection in pregnancy. In the right anterior mammary gland was a 3 mm. nodule, hard, white, with attached cyst; it was not ulcerated. On the right side anterior to the posterior mammary gland is another small white nodule, about 3 mm. in diameter, but not cystic. There are no other tumor growths or metastases, and the kidneys show no abnormalities.

The anterior growth consists of a mass of oval shaped cells, closely packed together, except for the presence of several cystic cavities. It is distinctly within the mammary gland and has evidently grown by expansion rather than infiltration, although there is evident invasion of the capsule by tumor cells in many places. The cells are somewhat smaller and less elongated than the cells in the tumor of 3383, and the nuclei have less dense chromatin. Occasional mitotic nuclei are seen. The cysts have no definite lining, beyond that furnished by the tumor cells which lie at the surface without forming a distinct membrane. Nevertheless, in the substance of the tumor are occasional collections of cells, not essentially different from the spindle or oval cells of the tumor, which are grouped to form more or less complete tubules. These have no basement membrane, but lie in contact with the spindle cells in such a way as to suggest the so-called "rosettes" of retinal gliomas or neurocytomas.

The smaller nodule has exactly the same structure as the tumor in 3383 (fig. 10). Hence these two tumors in 3413 are somewhat different from each other, but both have the same fundamental character of showing elements resembling those of a spindle-cell sarcoma and tubular structures suggesting an adenomatous nature.

11939. A female mouse had a tumor in the left subaxillary region that seemed to be one of the usual mammary gland carcinomas. It measured 20 by 18 by 18 mm. The lungs, mediastinal tissues, and chest wall were riddled with small tumor nodules.

Microscopically, the primary tumor consists of varying structures. In large part it is composed of solidly packed cells arranged in alveoli, into which connective tissue fibrils do not penetrate, thus resembling carcinoma, especially in that the cells nearest the stroma are frequently cuboidal. The cells are of medium size, polyhedral, with deep staining but somewhat vesicular nuclei, and a moderate amount of finely granular cytoplasm. The stroma is extremely scanty, and the alveoli for the most part seem to have but little between them except blood channels in which the walls show an endothelial lining only in some places; to a large extent these blood spaces seemed to be composed only of tumor cells. The alveolar tissue with polyhedral cells passes in many places without observable transition into masses of spindle cells, which, except in shape, resemble the polyhedral cells. This latter sort of tissue constitutes about half the primary tumor, in some fields being practically pure, while other areas of equal size show only the alveolar structure, although most of the tissue shows a mixture of both types. In the spindle-cell areas the blood channels are especially numerous, and seem to be composed solely of tumor cells, thus resembling a sarcoma. Mitotic figures are very numerous in all parts, and the characteristic multipolar and asymmetrical mitoses of cancer are abundant. The tumor infiltrates freely, and shows some central necrosis.

The numerous metastatic growths show both types of tissue. In the lungs, they are mingled in about the same proportion as in the primary growth; in the mediastinum, the spindle-cell type predominates, although some cuboidal- and polymorphous-cell elements appear; in the chest wall, where the ribs are eroded by tumor tissue, the cells are even more of the spindle cell type.

An interpretation of such a tumor as this is difficult. It corresponds to the growths sometimes described as carcinoma sarcomatodes or as sarcocarcinoma, but it is difficult to be certain that both cells are, after all, not merely modifications of cells of one origin. Ewing (17) has recently taken this position, and Dr. E. R. Le Count, who examined the sections from this case, states that he would prefer to defend the position that the growth is entirely carcinomatous than that is consists of both sarcomatous and carcinomatous elements. Dr. Ewing also holds the same view after examining our sections.

MEDIASTINAL "LYMPHOSARCOMAS"

An interesting type of growths has been found arising in the mediastinum of eleven mice in this series, which seems to correspond closely to the characteristic neoplasms observed in the same location in man, and usually designated as lymphosarcoma. Similar mediastinal neoplasms have been described in mice by others. Thus, Tyzzer (18) described four tumors arising in the thymus among ten cases of lymphomatous tumors, and these correspond closely to our material in many respects. On account of the great uncertainty concerning the nature of the lymphomatous growths in mice, and as a special study of them as a group is under way, they are not included in this series of sarcomas, and will be described only briefly.

Five were in female mice, six in males. The typical cases show the superior mediastinal space filled with a white, yellowish white or slightly pinkish tissue, soft, and apparently arising from the site of the thymus. Sometimes the growth seems to be encapsulated but expansile, crowding the lungs apart, pushing down or spreading over the heart; less often it is infiltrative and adherent to the adjacent chest wall, pericardium and other structures. In all cases the growth surrounds and infiltrates the trachea, and spreads along the bronchi and great vessels into the hilum of the lung where it forms sheaths of tissue about these structures for some distance into the lung. Usually the pericardium, mediastinal fatty tissues, esophagus, and often the myocardium, are infiltrated by the tumor cells. In contrast to leukemia and pseudoleukemia, which often also cause lymphoid growths in the mediastinum, the spleen, liver, kidneys, and the systematic lymph glands do not exhibit lymphoid hyperplasia or lymphomatous nodules; where such a diffuse infiltration does occur, we have not included the case, as the distinction from pseudoleukemia is too difficult. However, leukemia and pseudoleukemia, although they cause some lymphoid hyperplasia about the bronchi and great vessels of the lung, we have not found to result in the formation of such great cellular masses as are seen in these mediastinal growths. In many respects these resemble the "thymomas" as described by Ewing (19) in a recent article, especially as to their origin which is apparently from the thymus, or at least from its site.

Microscopically these tumors are all composed of dense masses of round cells, but there are considerable variations in the types of cells included under this description. In some cases the cells are much larger than lymphocytes with a small amount of cytoplasm staining well with eosin, and a relatively pale, semivesicular nucleus. In others the cells approach more closely to the lymphocytes, but always larger than normal lymphoid cells. Intermediate types occur, and there may be some admixture of different types of cells, although usually one type of cell predominates greatly. The cells are too closely packed together for any definite arrangement although a delicate reticulum divides them up into pseudo-lobules in some places. Extensive infiltration into all adjacent tissues is always seen, although mitotic figures are scanty in most cases; they are most abundant in the larger cell types. The cells invade freely the trachea, esophagus, pericardium, myocardium, and especially the mediastinal areolar tissue; in the lungs they spread luxuriantly along the bronchi and large vessels but do not infiltrate much into the adjacent lung tissue. Often large hyaline cells with one or more nuclei are present, resembling certain cells described in tumors arising in the same location in man. In two only were structures found that seemed to be remnants of Hassell's corpuscles, but in one of these this tissue was abundant with some "cholesterol slits" and other evidences of degeneration. Necrosis was seldom seen in these tumors, and then not extensive, nor was hemorrhage common.

METASTASIS

We have not yet tabulated the frequency of metastasis of carcinoma in the Slye stock, but Murray reports about 50 per cent of spontaneous carcinomas with metastasis in his material. In the 87 mice of this series 23 showed metastatic tumors, or 26.4 per cent. The height of this figure is largely caused by the osteosarcomas, which accounted for 9 of the 27. Taking up the type of sarcoma with the number giving rise to metastases, we have

Osteosarcomas	9 of 12 or 75
Spindle-cell	6 of 47 or 13
Polymorphous-cell	3 of 10 or 30
Oval-cell	0 of 3 or 0
Round-cell	4 of 10 or 40
Alveolar	1 of 2 or 50
Angiosarcoma	0 of 3 or 0
Total	23 of 87 or 26.4

We find that, as in human sarcomas, the spindle-cell sarcomas are much less likely to produce metastases than the polymorphous-cell or round-cell types, and those spindle-cell sarcomas that produced metastases were mostly of the larger cell types (fig. 11). The fibrosarcomas rarely produce metastasis. In some cases, especially with the osteosarcomas, multiple metastases were found. The distribution of the metastases was as follows: Lung, 13; liver, 10; lymph glands, 5; mediastinum, 3; chest wall, 3; spleen, 2; pleura, 2; diaphragm, 2; retroperitoneal, 2; kidney, 1. These figures refer solely to the number of times these organs were found the seat of metastasis, in many cases there having been many secondary growths in a given organ. It will be noted that the sites of metastasis correspond closely with what is observed in human sarcoma, the lung and liver being most often involved, and then the lymph glands.

ETIOLOGY

As with human tumors, mouse sarcomas frequently arise at the site of a trauma. This has been observed in eleven of this series. It is, of course, evident that we have no knowledge of how many of the other mice had received injuries at the point at which they subsequently developed a sarcoma, for the life of a mouse is beset with many accidents and deeds of violence. Especially among the males, wounds are often received in fighting. In the eleven cases in which the relation was clear, the injury was noted and afterward the sarcoma made its appearance at this point, or else an early tumor was observed at the site of a scar from some old injury. A particularly good example is furnished by case 3117, previously described under the heading of "multiple primary sarcoma." This mouse was bitten on the back and genitals, and so severely wounded that it was taken to the "hospital" to recover. There, while under observation, two typical spindle-cell sarcomas arose at the site of these wounds.

The influence of heredity in determining the occurrence of sarcoma in the site of old wounds has been especially noted in this series, and found to be important. This is a large subject, and will constitute by itself a separate paper, and, hence, will not be discussed here. Also the relation of age to tumor formation requires more detailed study than we have yet been able to give it, and is reserved for future consideration. We have observed no relation of the sarcomas to any particular form of infection or inflammation. In not a single instance have we observed any parasites in or about the sarcoma. This is of particular interest in view of the relation of liver parasites to sarcoma of the liver in rats (20). Our mice often have tapeworms filling up the bile ducts and leading to extensive abscesses of the liver, but we have never observed either sarcomas or liver adenomas arising in these lesions. There seems to be no relation between these sarcomas and the leukemias; at least in this series we had only two cases in which sarcoma co-existed with leukemia and none with pseudoleukemias. The tendency to the co-existence of tumors is, however, quite marked, as with all other types of spontaneous mouse tumors yet studied, and this may be interpreted as the existence of a high natural susceptibility to the formation of neoplasms in the affected animals. It is certain that the more highly cancerous the ancestry of mice, the more likely they are to have multiple independent spontaneous tumors.

SUMMARY

In a series of 12,000 autopsies on the bodies of mice dying at all ages, either from natural causes or in a relatively small proportion from accident, there were found 87 mice with neoplasms meeting all the criteria of sarcoma. These do not include any growths of the character of lymphosarcomas, because of the recognized uncertainly of the nature and diagnosis of these neoplasms; also we have excluded eleven cases of characteristic mediastinal tumors, arising at the site of the thymus and infiltrating the lungs. Tumors of the testicle, adrenal, ovaries and kidneys of "mesothelioma" character have also been omitted.

Spindle-cell sarcomas constitute over half the tumors, there being 47 of all types, not including 3 oval-cell sarcomas, 3 perivascular sarcomas, and two alveolar sarcomas. There were 12 osteoid sarcomas, and 10 polymorphous-cell, and 10 round-cell sarcomas. Metastasis was observed in twenty-three cases, or

26.4 per cent, the osteoid sarcomas leading with 75 per cent, metastasis occurred in only 13 per cent of the spindle-cell sarcomas. Lungs, liver and lymph glands showed most of the metastases. In all respects these sarcomas of mice correspond with the sarcomas of men, although we have found no examples of melanosarcoma, multiple myelomas, or myeloid sarcoma. In at least eleven cases the sarcomas definitely arose at the site of previous injuries. In a few instances there seemed to be two distinct primary sarcomas in the same mouse, and there were three instances in which the growths suggested a mixture of sarcomatous and carcinomatous elements. About half the sarcomas arose in the subcutaneous tissues, apparently from the mammary gland in most cases; and next in frequency from the osseous tissues. Two cases of sarcoma of the uterus were observed, the only uterine tumors of any kind observed in all the autopsies. Twenty of the sarcoma mice also had other independent tumors, lung tumors being most numerous. Two mammary gland tumors were found closely resembling in structure the embryonal adenosarcoma of the kidney of man and other animals, but without renal involvement. The influence of inheritance on the incidence of sarcoma has been found to be marked, but is reserved for further discussion.

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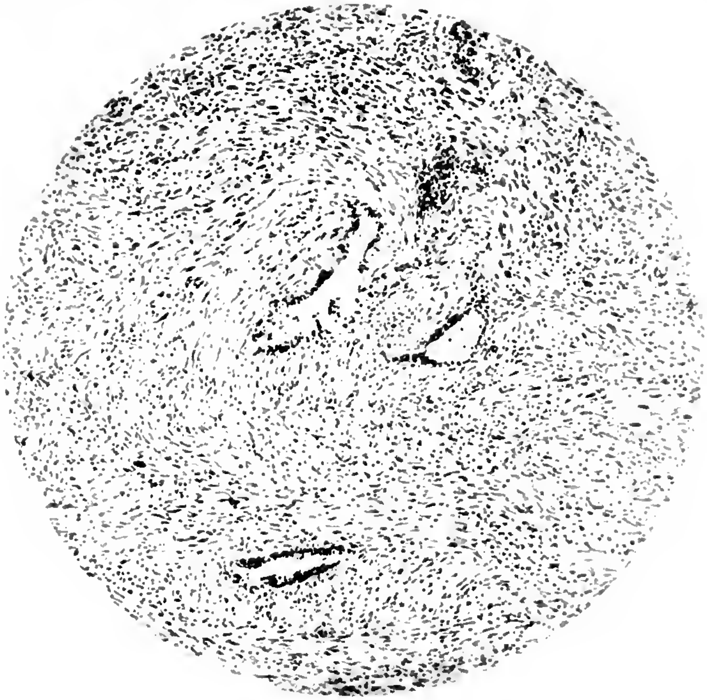
PLATE 1

Fig. 1. Pericanalicular fibrosarcoma of the mammary gland. × 120. The tubules are lined with epithelium and are scanty.

Fig. 3. Squamous-cell carcinoma of the mouth, under which is a tissue resembling a spindle-cell sarcoma, apparently arising in the periosteum of the lower jaw. Some fields show many giant cells. The bone, in the center of the field shown, is eroded and invaded by tumor tissue. × 70.



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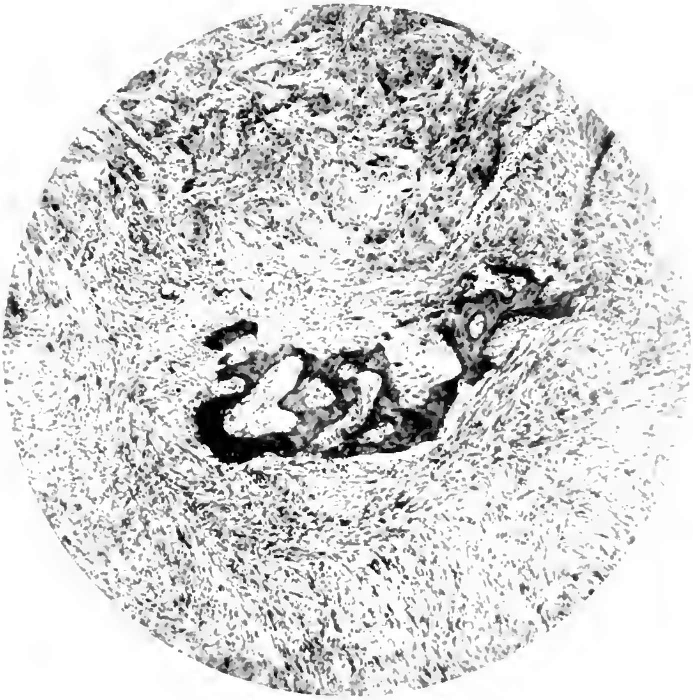

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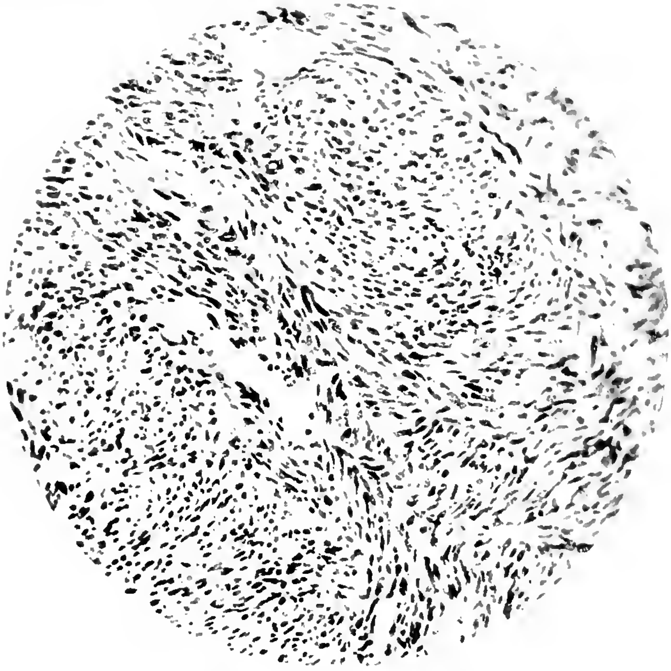
PLATE 2

FIG. 4. Spindle-cell sarcoma, arising in subcutaneous tissue, shows typical spindle-cell character, exhibited by the entire tumor. × 100.

FIG. 5. Large spindle-cell sarcoma arising in periosteum of femur, showing many small giant cells, also muscle cells infiltrated by the tumor tissue. Metastasis from this tumor in lung shown in figure 11. × 120.



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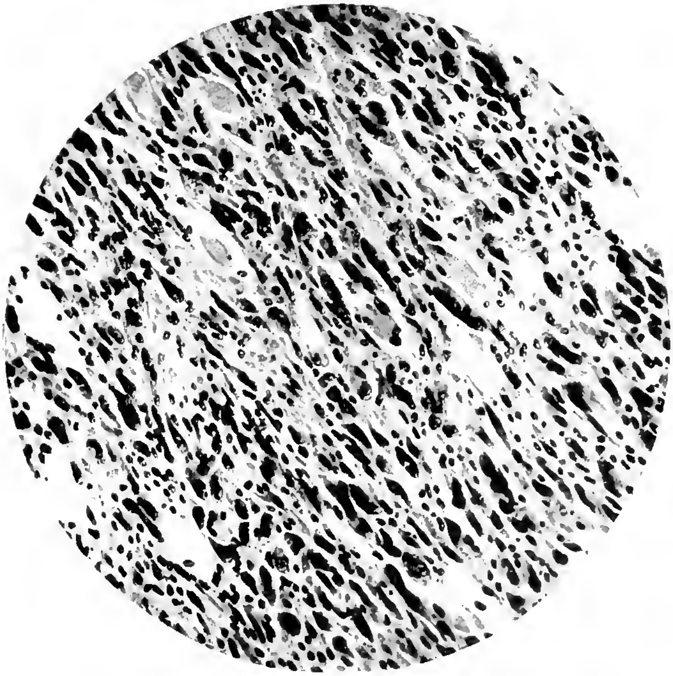

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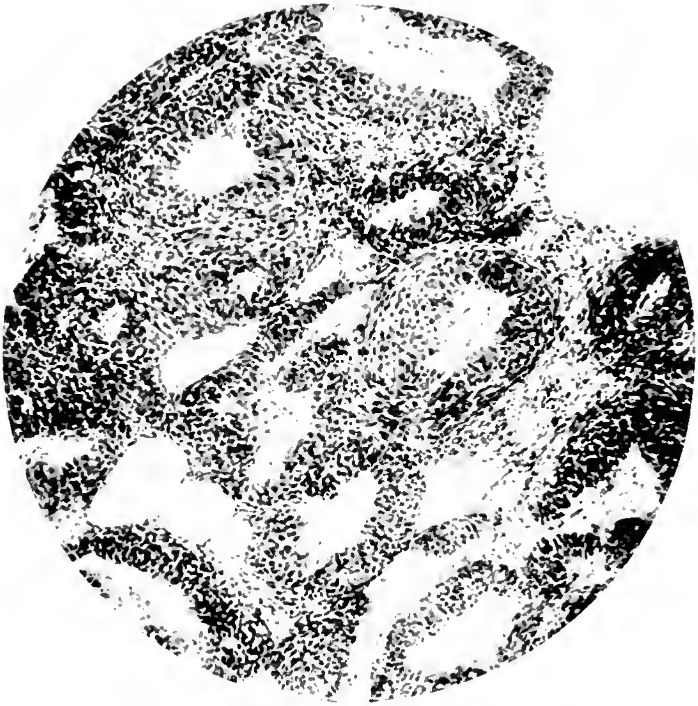
PLATE 3

Fig. 6. Angiosarcoma arising in subcutaneous tissue. × 110.

Fig. 7. Osteoid sarcoma metastasis in liver, secondary to tumor in femur. Shows central calcification and atypical ossification, with more active cellular growth at the periphery. × 60.



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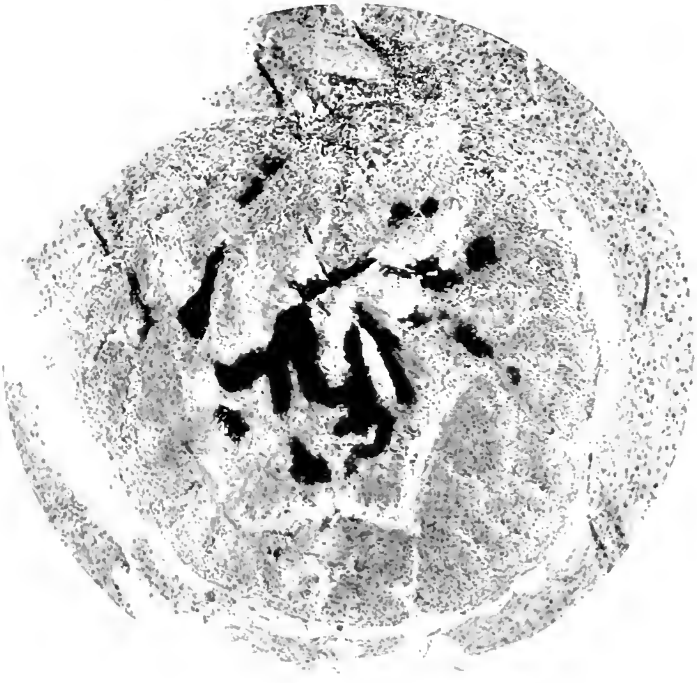

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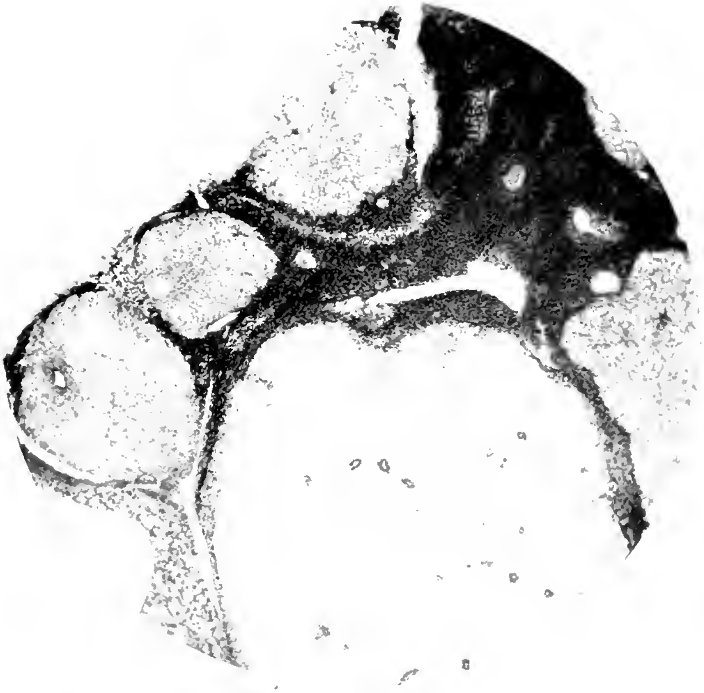
PLATE 4

FIG. 8. Nodular areas of fatty tissue in liver of mouse with an osteoid sarcoma. In other parts of this liver tumor metastases are found. Note how well defined these areas are, and the absence of fatty changes in the liver tissue between them. × 24.

FIG. 9. Secondary osteoid sarcoma nodule in the liver, surrounded by a tissue resembling fatty marrow. All stages between those illustrated in figures 7 and 8 can be found. × 95.



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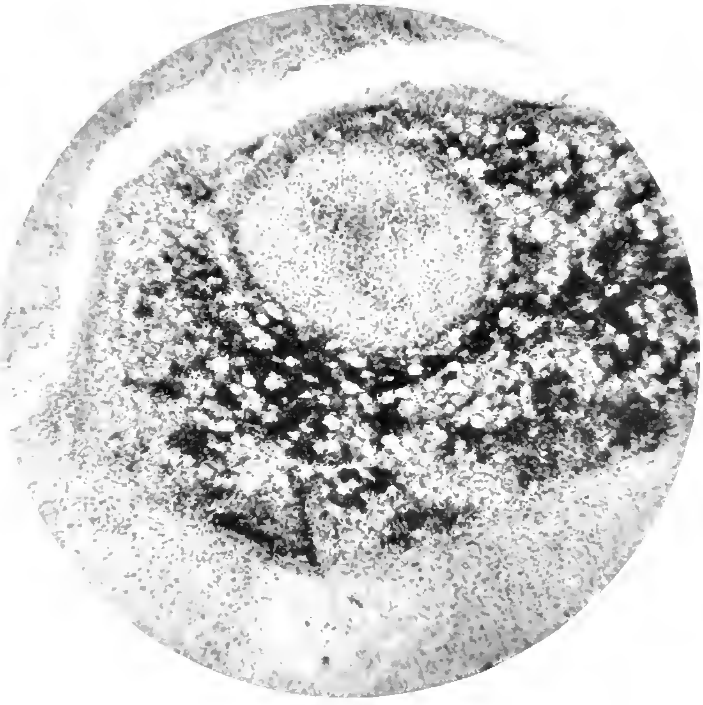

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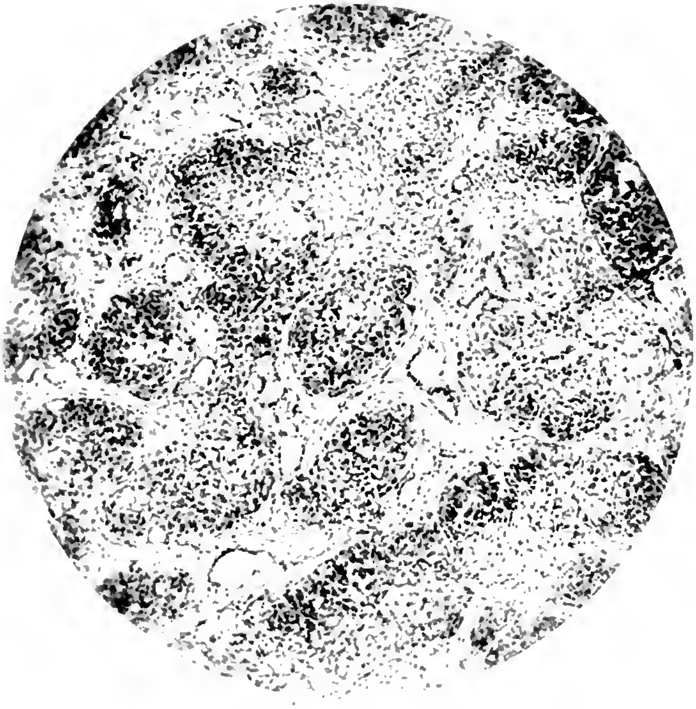
PLATE 5

Fig. 10. Mammary gland tumor resembling in histology the embryonal adenosarcomas of the kidney, although there was no renal tumor in this case. The islands of spindle cells show some mucoid degeneration. × 130.

Fig. 11. Secondary spindle-cell sarcoma in the lung, adjacent to a large bronchus. Primary growth was in the femur, and shown in figure 5. × 120.



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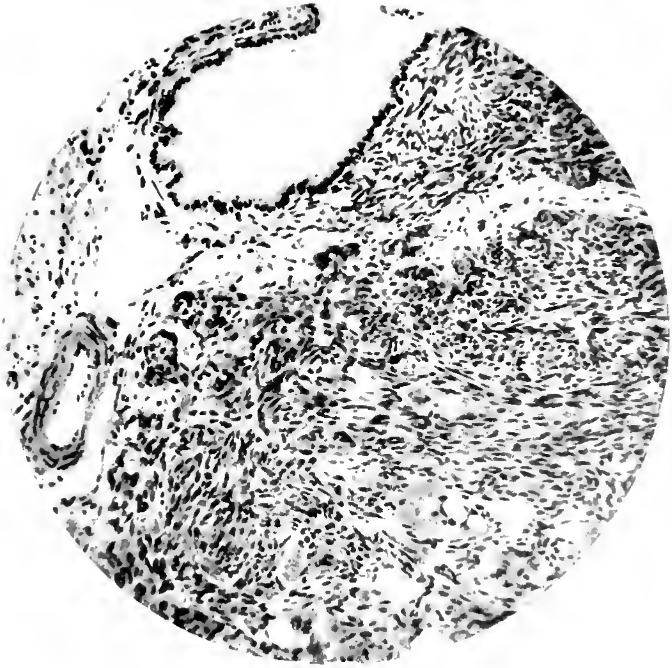

11

SPONTANEOUS TUMORS OF THE RAT

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Although spontaneous tumors of the rat have ceased to be a pathological novelty, the number of cases occurring in this species is, nevertheless, small as compared with that of neoplasms in the mouse. In the experience of McCoy (1), the rate of incidence, considering benign and malignant growths collectively, was only about one in one thousand, and Woolley and Wherry (2) have reported a rate not much higher. Yet it must not be forgotten that the tumors recorded by these authors occurred in wild rats, of which probably fewer survive to reach the cancer age than is the case with white mice.

A survey of the cases thus far described in rats shows rather striking variations from the common type of mouse tumor. Thus, of 123 cases (table 1) gathered from the literature, 74 (60 per cent) were malignant; of these only 18 (14 per cent) were of epithelial origin. Malignant tumors of connective tissue origin, comprising 86 per cent of all malignant growths, arose in the liver in 53 per cent of the cases, and in 90 per cent of these the *Cysticercus fasciolaris* was demonstrable in the tumor. Six per cent of the benign growths were of epithelial origin, 47 per cent of the connective tissue type, and 47 per cent of the mixed epithelial and connective tissue variety. Of the benign tumors, 69 per cent occurred in the breast. The sex of the animal was recorded for 114 animals, of which 75 per cent were females.

It is interesting that the predominating malignant tumor should be of the connective tissue type, and that the liver should be the organ most frequently involved; for in the mouse, carcinomata occur much more frequently than sarcomata, and the breast is the organ generally attacked. The liver, on the other hand, is relatively seldom the seat of connective tissue growths in mice (according to the data of Slye, Holmes, and Wells (3) no cases were found in 10,000 autopsies), and notwithstanding the fact that the *Cysticercus fasciolaris* is almost as common a parasite in the liver of the mouse as of the rat, a hepatic sarcoma attributable to this organism has never been reported in the mouse.

The following table summarizes 123 cases of tumors of the rat which we have been able to gather from the literature:

TABLE 1

Benign tumors:		
Fibroma:		
	Breast	19
	Liver	2
Lipoma:		
	Subcutaneous	1
Angioma:		
	Liver	1
Fibroadenoma:		
	Breast	15
	Uterus	2
	Kidney	7
Papilloma:		
	Bladder	1
	Kidney	1
Malignant tumors:		
Sarcoma:		
	Subcutaneous	11
	Liver	30
	Thyroid gland	2
	Submaxillary gland	1
	Bone	3
	Mesentery	7
	Adrenal gland	1
Carcinoma:		
	Breast	5
	Seminal vesicle	1
	Kidney	8
	Bladder	1
Epithelioma:		
	Tongue	1
	Vulva	2
Endothelioma:		
	Peritoneum	1

From December 1913, to October, 1915, there were received at this laboratory approximately 15,000 rats, the vast majority of which were comparatively young animals from three to eight months old. Four of the total number developed tumors, three of the bearers being full grown and one a young rat (nos. 3 and 6 of the cases here described, and two others previously reported (4)). The tumor incidence in these young animals was thus 1 in 3750. Since October, 1915, 4300 adult rats of mixed stock from different dealers have been received at the laboratory; of these animals, 21 developed tumors (table 2), an incidence of approximately 1 in 200, and a much higher rate than any previously recorded. Three of the 21 animals had multiple tumors. Of the growths here reported, 78 per cent occurred in females. Nineteen per cent of the neoplasms were malignant, of which 33 per cent were carcinomata. The benign tumors were of the mixed connective tissue and epithelial type in 16 per cent of the cases, and of the pure epithelial type in 80 per cent.

The following table summarizes the 32 tumors discovered in our own material:

TABLE 2

Benign tumors:			
	Fibroma:		
		Subcutaneous	1
	Adenofibroma:		
		Breast	5
	Adenoma:		
		Kidney	10
		Thyroid	2
	Cystadenoma:		
		Thyroid	3
	Papillary cystadenoma:		
		Thyroid	5
Malignant tumors:			
	Carcinoma:		
		Breast	2
	Sarcoma:		
		Liver	4

In the following paragraphs, a general description of the breast tumors is recorded.

Rat 3, an old male, bearing an adenofibroma of the breast measuring 2.5 by 4 cm., in the left inguinal region. An unsuccessful attempt was made to transplant the growth.

Rat 6, an old female with a mammary fibrocystadenoma in the left hypogastrium, measuring 3.5 by 2 cm., and resembling the tumors of this type found in the human subject. Transplantation was not attempted.

Rat 11, a young female with a fibroadenoma (fig. 1) measuring 3 by 3.5 cm., in the right axilla. No transplantation was attempted.

Rat 13, an old female presenting a tumor 2.3 by 3.3 cm. in the left groin. The tumor was a fibroadenoma containing about equal parts of fibrous and glandular tissue (fig. 2). Transplantation was unsuccessful.

Rat 19, an old female with a large tumor in the right groin. The growth was a typical fibrocystadenoma (fig. 3) with actively secreting cells. In some areas the connective tissue predominated over the glandular elements. This growth was not transplanted.

Rat 10, a full-grown female with a tumor measuring 1.8 by 2.4 cm., in the left flank. The neoplasm contained solid alveoli (fig. 4) and alveoli with several or many lumina, separated by thin bands of cellular connective tissue. In those areas where the connective tissue preponderated, the cells were arranged in strands or larger cords, many of the latter containing multiple lumina. Some of the solid alveoli showed central necrosis. The individual tumor cells exhibited considerable variation in shape and size, and mitotic figures were abundant. Included in the parenchyma of the tumor were portions of sebaceous gland, but no squamous epithelium was found. No metastases were demonstrable. The tumor was successfully transplanted and is now in the fifth generation; sebaceous elements have been found in each generation.

Rat 20 an adult female with a tumor measuring 2.7 by 3.2 cm., situated in the right inguinal region and extending beyond the middle line of the body, displacing the clitoris and surrounding the anus. The alveoli composing the tumor were made up of concentric rows of cells arranged about a central lumen (fig.

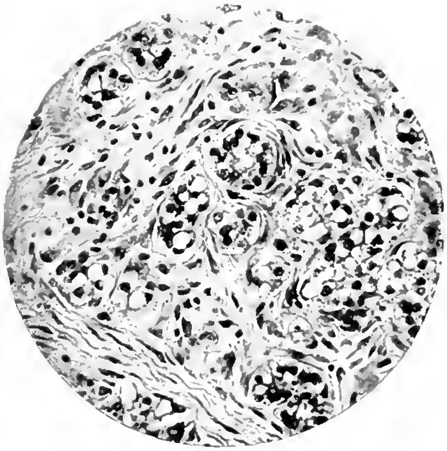

FIG. 1. Fibroadenoma of breast, Rat 11

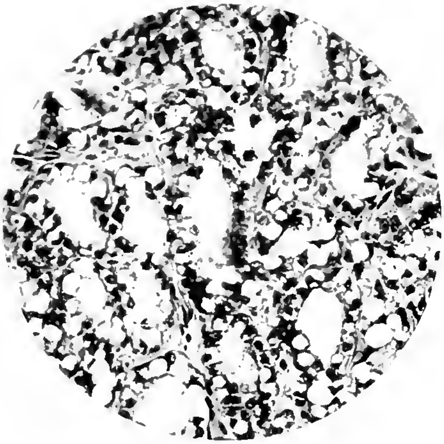

FIG. 2. Fibroadenoma of breast, Rat 13

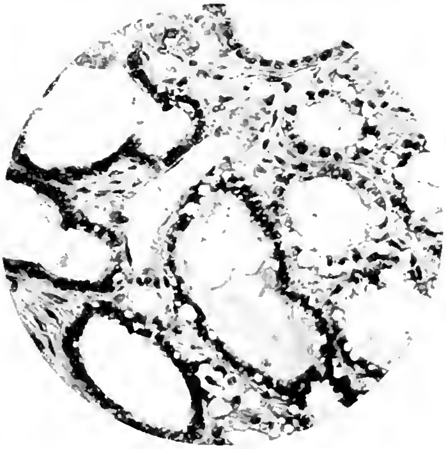

FIG. 3. Cystic fibroadenoma of breast, Rat 19

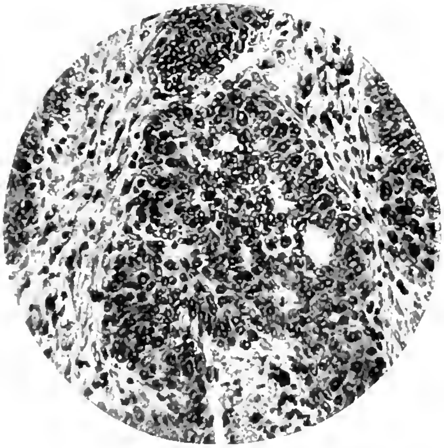

FIG. 4. Carcinoma of breast, Rat 10/0

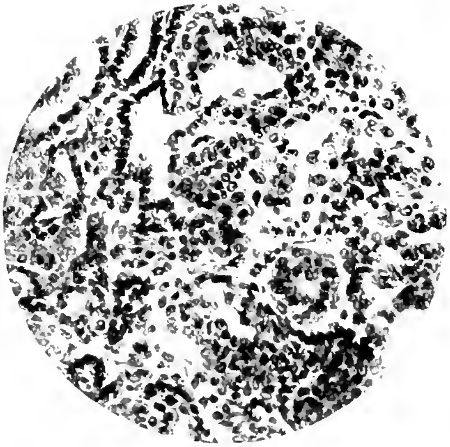

FIG. 5. Carcinoma of breast, Rat 20/0

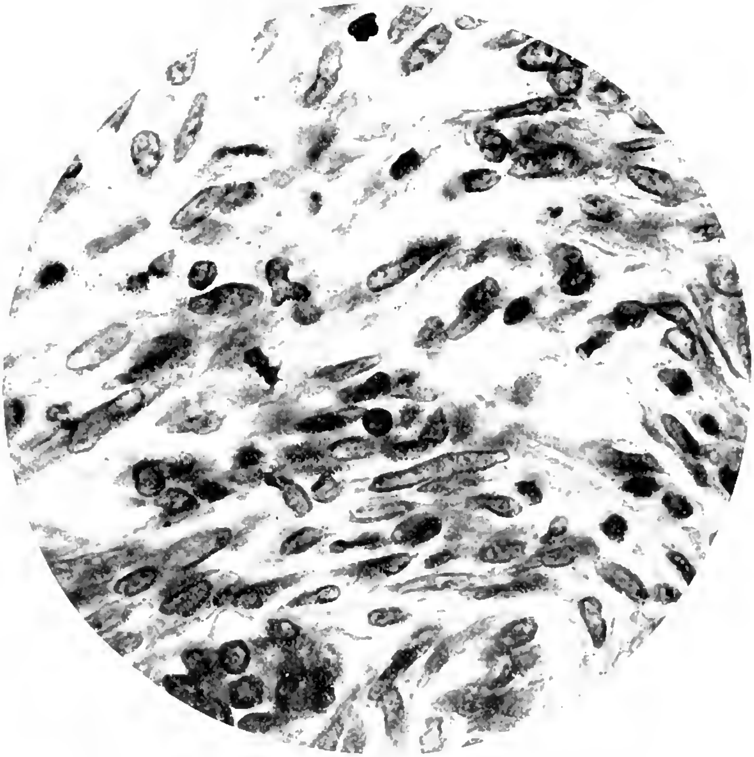

FIG. 6. Stroma of tumor in Rat 20 showing active proliferation; 6 mitoses in the field.

5); the cells were flat or cuboidal, becoming larger and polyhedral in shape toward the periphery of the alveolus. Mitotic figures were common. Scattered freely among the alveoli were hyperplastic breast ducts and acini, which in places were adenomatous. The tumor was divided into irregular lobules by fairly dense bands of connective tissue, the alveoli being separated from one another by delicate strands of connective tissue; in some areas, the connective tissue was very cellular and actively proliferating (fig. 6); in others, scanty. Transitions between duct epithelium and tumor were demonstrable. The contrast in staining qualities between the neoplastic elements and those of the ducts and acini was striking, the latter taking a more intense stain. No metastases were found. The tumor was successfully transplanted and is now in the second generation.

The following tumors, occurring in the kidney, were all adenomata.

Rat 12, an old male, the bearer of multiple growths, each kidney having from four to five tumors. The largest, a papillary cystadenoma measuring 3 by 3 mm., was situated on the anterior surface of the right kidney at the lower border of the hilus; it was elevated above the surface (fig. 7) and extended a few millimeters into the cortex. On microscopical examination the tumor presented cystic spaces with papillary ingrowths, or partly filled with plugs of tumor cells; the cells were cuboidal or low cylindrical in shape and generally uniform in size. Mitotic figures were few in number. The cells covering the papillæ were arranged, as a rule, in a single layer, though occasionally several were encountered. The blood supply of the tumor was good and the stroma scanty. The following is a brief description of the other renal tumors: Two growths of the same general type as the one just described, but of microscopic size, were found, one in each kidney. Another minute tumor (fig. 8) was composed of polygonal cells packed closely together and varying greatly in size; there were a few giant cells with large single nuclei. Blood capillaries could be made out between the cells, but no definite stroma was distinguishable. Areas of degeneration were common.

A third type of renal neoplasm discovered in this rat was composed of small acini with a single row of cuboidal cells, separated by strands of connective tissue. Still another growth, in which the cells resembled those of a polyhedral cell adenoma, presented in general an alveolar arrangement; some of the alveoli contained lumina. In certain areas the tumor was distinctly acinous, with large cells, some of which stained like the surrounding normal cells of the kidney, while others resembled in their staining properties the cells of the adrenal. All the adenomata just described were in the cortex of the kidney, though two of them extended into the medulla.

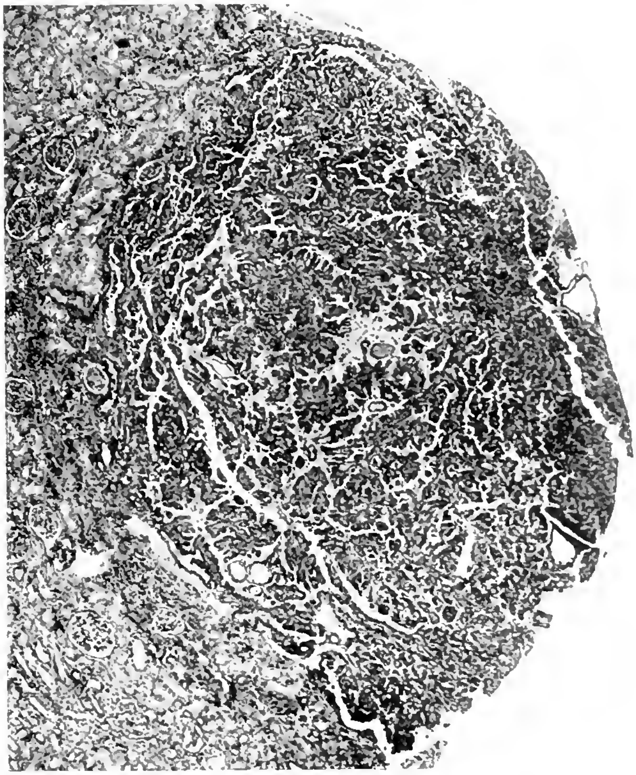

FIG. 7. Papillary cystadenoma of kidney, Rat 12

An attempt to propagate the largest renal tumor was unsuccessful.

This rat also had a tumor on the right side of the neck, measuring 1 by 1.5 cm. This was a fibroma (fig. 9), which contained relatively large quantities of collagen undergoing hyaline degeneration.

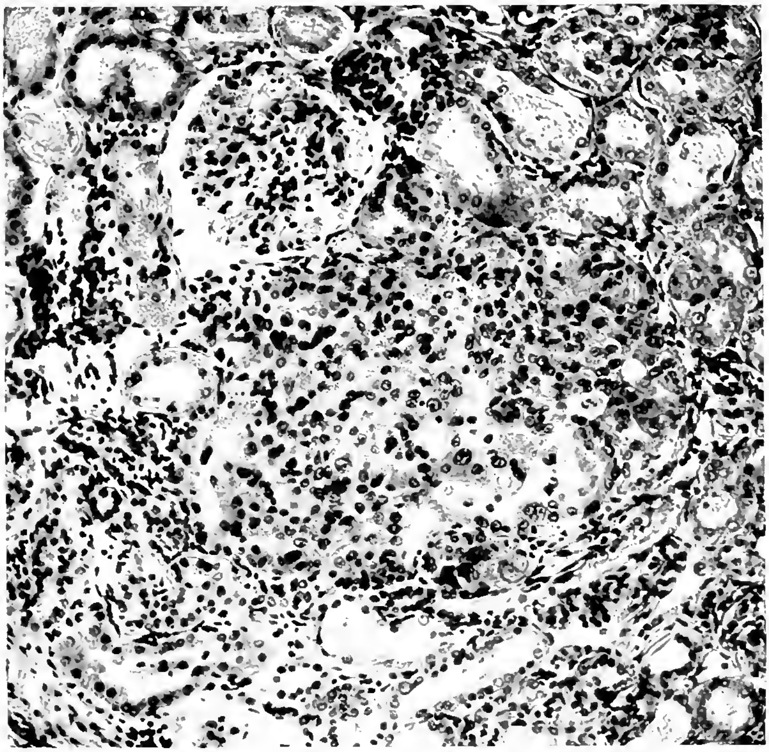

FIG. 8. Polymorphous adenoma of kidney, Rat 12

Rat 28. The tumor was found at autopsy in an old animal, the sex of which was not recorded. The growth, which measured a trifle less than 1 mm. in diameter, occurred in the cortex of the kidney, directly beneath the capsule; it consisted of solid alveoli separated by delicate strands of connective tissue and capillaries. The cells of the alveoli were large and polyhedral, with a deeply

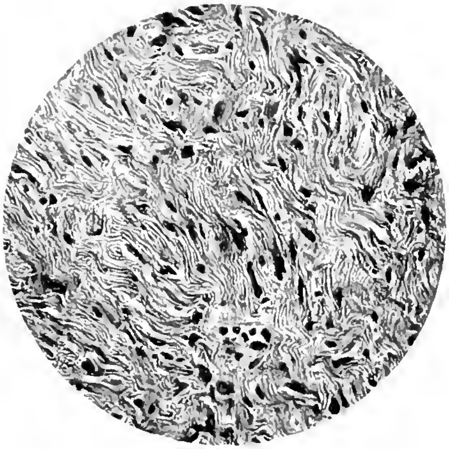

FIG. 9. Fibroma of neck, Rat 12

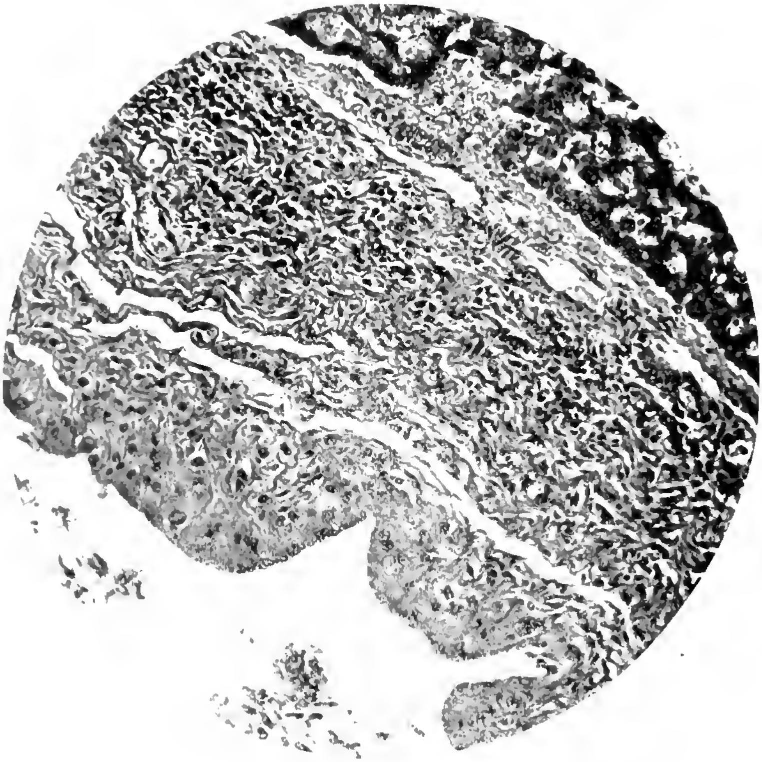

FIG. 10. A typical cyst wall of the *Cysticercus fasciolaris*

staining protoplasm. Multinuclear and mononuclear giant cells were present. Certain of the alveoli were undergoing fatty degeneration and necrosis in their central portions. No mitotic figures were observed. The tumor was encapsulated and smaller encapsulated tumor masses were found outside of the main capsule. No attempt was made to transplant the neoplasm.

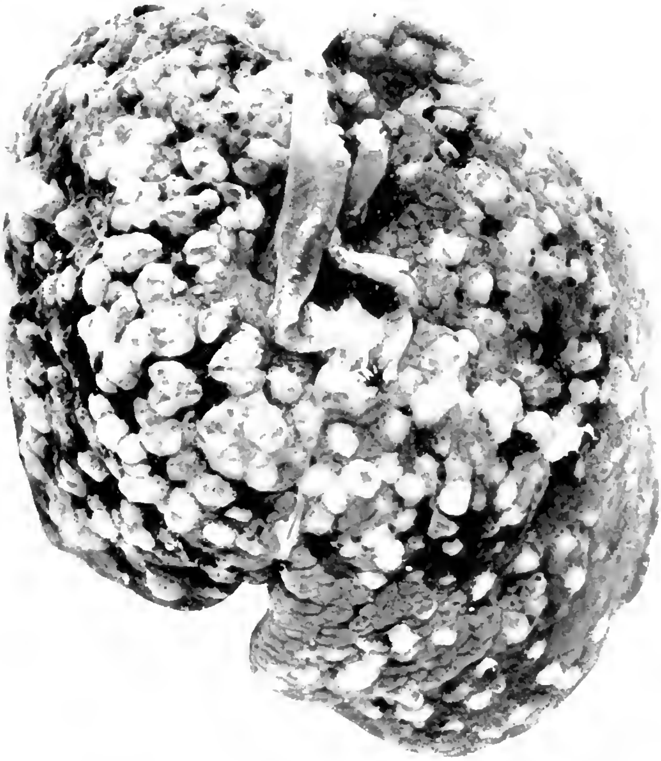

FIG. 11. Gross specimen of rat liver containing over 200 *Cysticerci fasciolaris*

These adenomata of the kidney are of interest because they present some of the distinguishing features of malignancy, such as marked variation in the size and staining properties of their cells; yet in all probability they are benign.

As indicated in table 1, primary malignant tumors of the liver are rather frequent in rats. Including those reported in this paper, six instances have come under our observation, all in connection with cysts. Those in the wall of cysts surrounding the Cysticercus fasciolaris are sarcomata of one of three types—polymorphous or spindle-cell sarcoma, or angiosarcoma.

The host of the adult parasite (*Tenia crassicollis*) is the cat, the cysticercus stage occurring in the liver of the rat or the mouse, where from one to several hundred parasites may occupy the organ (fig. 11); there they become surrounded by a connective tissue capsule formed from the stroma (fig. 10). Infection occurs through the ingestion of feces from infected cats.

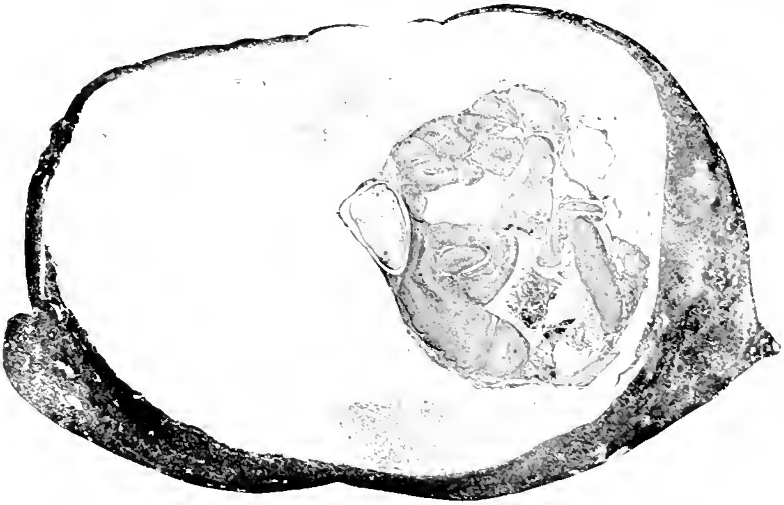

FIG. 12. Low power of entire tumor and cyst. Rat 15

It is to be emphasized here that 90 per cent of all the recorded sarcomata of the liver were associated with the presence of the *Cysticercus fasciolaris*. It is possible that in the remaining 10 per cent also the parasite was present, but remained undiscovered because not specifically sought for.

McCoy (1) states that the parasite is almost invariably dead when found in association with tumor formation, a statement which suggests that it has been found alive; in all the recorded instances where the condition of the parasite has been noted, however, it has been reported as dead.

We have been fortunate in having encountered sarcomata from their probable inception to the fully developed stage with innumerable metastases.

A description of the sarcomata of the liver is given in the following paragraphs.

Rat 15. In this animal, an old female, a large cestode cyst measuring 9 by 12 mm. was found at autopsy, attached to the dorsal surface of the left lobe of the liver, its ventral surface covered by liver while the remaining surface was bare. Over its ventral aspect the cyst wall and the liver tissue were converted into a greyish white translucent tissue suggesting a fibrous or malignant process. Microscopical examination of this portion of the cyst wall (fig. 12) showed active proliferation of the connective tissue elements with the production of two types of sarcoma: a polymorphous-cell tumor (fig. 13) originating chiefly from the cells lining the cyst cavity, and a spindle-cell growth

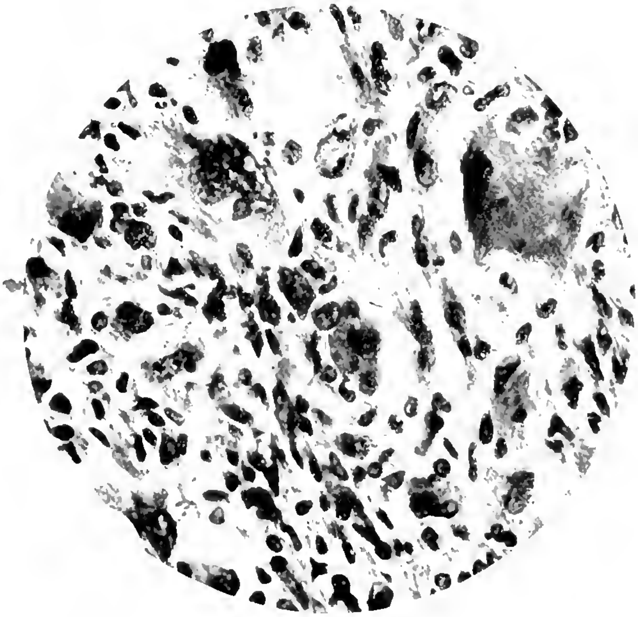

FIG. 13. High power of polymorphous-cell tumor, Rat 15

(fig. 14) arising from the cells in the outer portion of the cyst wall. The tumor was invading the liver. The neoplasm was associated with fibrotic and degenerative changes involving the cyst wall and a part of the overlying hepatic tissue. The cyst cavity contained a dead parasite.

On the surface of the right lobe of the liver was a second cyst measuring 4 by 4 mm. and containing a *live* parasite. The wall of this cyst (fig. 15) was unusually cellular, and, particularly in the small portion which was covered by a thin layer of liver, was in active proliferation. The cells lining the cavity, as well as those of the outermost part of its wall, were participating in the process, as was evidenced by the great number of mitotic

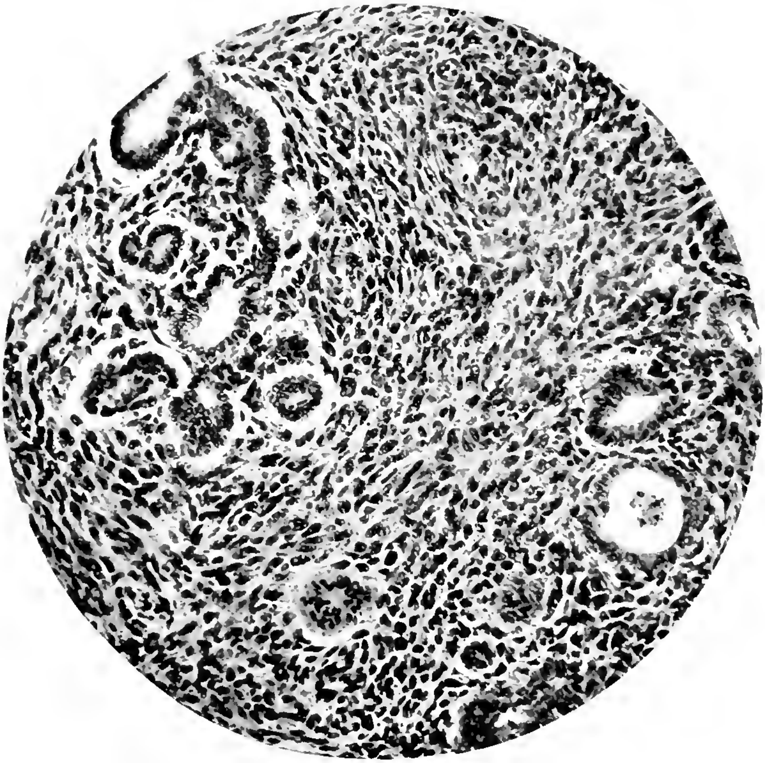
FIG. 14 High power of spindle-cell tumor. Rat 15

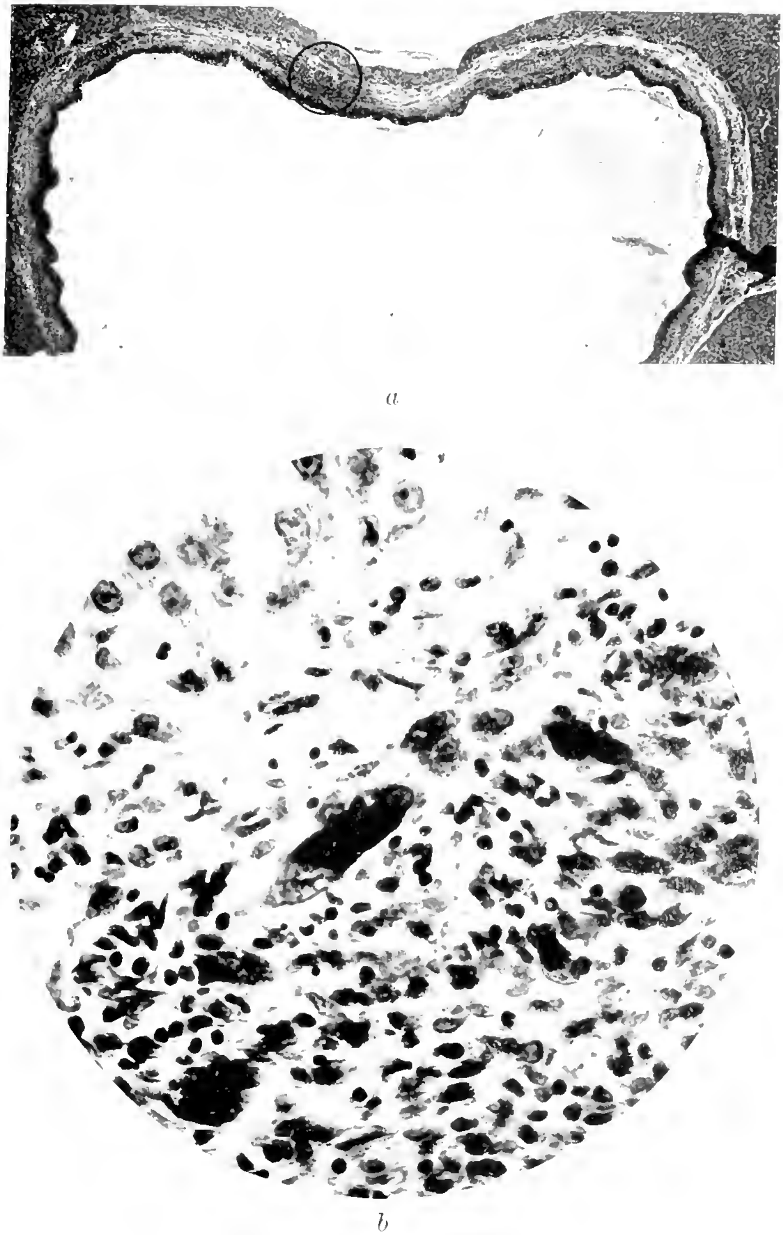

FIG. 15. *a*, Early sarcoma, Rat 15; *b*, high power of same

figures here discovered. There was considerable variability in the staining properties and in the size and shape of the cells: thus, although small spindle cells predominated, large round elements and spindle cells containing one or more nuclei could be found; a moderate number of small round cells also, and a few polynuclear elements were observed in this area. Nothing was found in the serial sections of the cyst wall to account for the proliferative activity of the connective tissue.

The tinctorial and dimensional irregularities, the large number of mitoses, and the lack of any evidence of infection or of the presence of any foreign substance capable of calling forth a connective tissue reaction, considered together with the presence of sarcoma in another region of the same organ, incline us to view the tumor as a very recent sarcoma originating in a susceptible tissue.

The presence of a live parasite in association with this early tumor suggests the possibility that in the tumors recorded by other authors the parasite may have been alive at the inception of the neoplasm (the death of the parasite occurring after the full development of the tumor possibly from interference with nutrition) or that dead parasites also are capable of producing malignant changes.

Neither of these tumors was transplanted.

The comparative infrequency of sarcoma of the liver, in contrast to the common occurrence in this organ of cestode cysts containing parasites measuring 30 to 40 cm. long, indicates that the development of a malignant growth depends on a condition of susceptibility resident in the animal itself as an intrinsic property of the organ as both Murray and Slye have suggested in another connection, and that a stimulus even more specific than the mere presence of the parasite acting for a long period of time (as is indicated by the size of the latter) is required to cause it to assert itself. That the presence of the parasite does not always induce malignancy even in susceptible animals is evidenced by Rat 22, an animal presenting four cysts of the liver, all of about equal size, only one of which showed malignant change. This indicates that the power to induce sarcomatous change in connective tissue, a causal relationship between parasite and sarcoma being assumed, is not a property common to all parasites irrespective of size.

Rat 17. This animal, a fully grown female, had multiple tumors in the liver, with involvement of the peritoneum and invasion of the diaphragm, stomach, and left kidney. Embedded in the left lobe of the liver, and surrounded by a wide zone of necrotic tumor, there was a cestode cyst of moderate size containing a dead parasite. The other tumors of the liver, which were more or less degenerated, were apparently of more recent origin, being probably metastatic. No cyst was observed in them. The tumor was a typical polymorphous-cell sarcoma.

A small papillary cystadenoma, with extreme thickening and hyaline degeneration of parts of its stroma, was found in the thyroid gland of this rat. Associated with this tumor there were chronic inflammatory changes in the larynx.

Neither of these growths was transplanted.

Rat 22. The tumor was found in an adult female during the course of a laparotomy. The liver contained four cysts, one of which, attached by a short pedicle to the ventral surface of the left lobe, showed signs of inflammation and great thickening of its walls; it contained a dead parasite. A portion of the cyst wall had been transformed into spindle-cell sarcoma (fig. 16) which was invading the liver. Beyond the tumor there was a wide zone of granulation tissue containing collections of plasma cells, some small round cells and eosinophiles. The plasma cells infiltrated the tumor tissue and an occasional mitotic figure was observed in them. Other portions of the cyst wall showed signs of considerable hemorrhage and were the seat of a small round-cell infiltration. The cyst cavity was partly filled with blood and granular débris.

The other three cysts showed no evidence of malignancy.

This is the second early tumor that we have encountered in which the parasite was dead. Vascular changes in the cyst wall secondary to degeneration and fibrosis may well account for the death of the worm, but whether the death of the parasite followed or preceded the appearance of the tumor it is impossible to say.

In a previous publication we stated that cystadenomata of the rat thyroid are extremely rare, but in view of the findings here recorded we are forced to modify that statement. In the 4300 rats of this second group, 9 tumors of the thyroid gland were found, a proportion of 1 to 478. The percentage would no doubt have been much higher if the thyroids of all these animals had been submitted to microscopical examination, since the majority of the neoplasms were not discovered in the gross, the glands having been excised solely because they were enlarged. Thus only enlarged thyroids, comprising a total of about 70 glands, were examined microscopically.

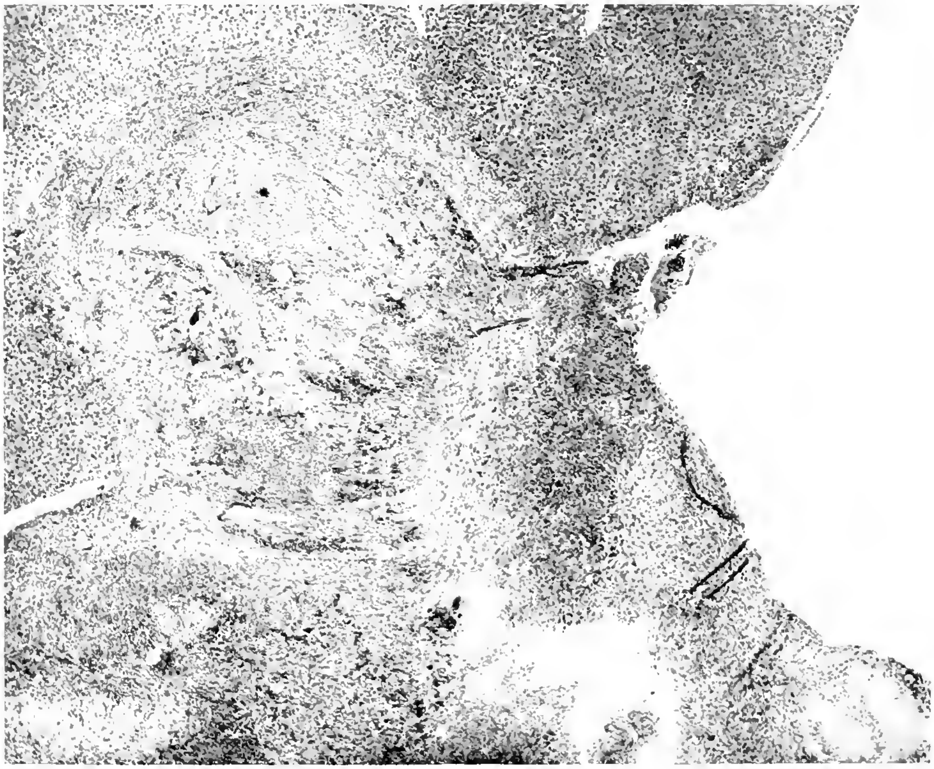

FIG. 16. Low power of tumor, Rat 22

The new growths of the thyroid may be classified as follows: adenomata (2), cystadenomata (2), papillary cystadenomata (6). They varied in size from microscopic growths to a tumor measuring 12 by 8 by 8 mm.

Rat 25 , an old female with a large tumor (fig. 17) occupying the position of the left lobe of the thyroid, and presenting a composite picture of adenoma, cystadenoma, and papillary cystadenoma. The individual cells were cuboidal, low cylindrical, or flat, and were arranged in single rows lining the cysts (which contained a colloid material), covering the papillae, or in acini, or compact masses without definite acinous structure. They stained deeply with hematoxylin and showed some variation in

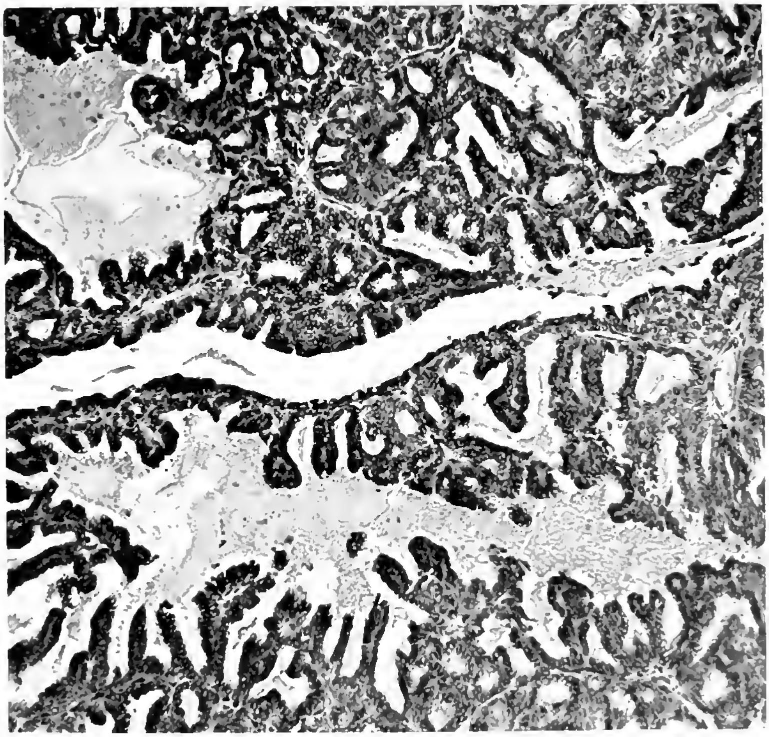

FIG. 17. Tumor of thyroid, Rat 25

size; mitotic figures were few in number. The delicate stroma was well vascularized.

The larynx and the tissues surrounding the tumor were the seat of a moderate grade of chronic inflammation.

The tumor was transplanted into 84 animals without success. A detailed description of the other growths is omitted, as each was typical of its class.

An interesting feature observed in all the rats with thyroid tumors was a chronic inflammatory condition of the larynx, which often involved the perithyroid tissues. This lesion varied in degree from a mild inflammation of the mucosa and the submucosa, confined chiefly to the region in which are located the serous and mucous glands of the larynx, to a severe condition affecting to some extent the greater part of the larynx and the thyroid gland. Metaplasia of the laryngeal epithelium, with the production of stratified epithelium, was occasionally present.

Although this inflammatory condition is not confined to tumor-bearing rats, being common also in those without tumors, its existence may be correlated in some way, perhaps, with the occurrence of neoplasms in the thyroid gland.

CONCLUSIONS

The incidence of spontaneous tumors in white rats is higher than in wild rats, partly due, in all probability, to the greater longevity of the white rat.

Sarcoma of the liver is much more common in white rats than in wild rats, probably because the former animals are kept in close confinement, and thus readily infected with the *Tenia crassicollis*. The cysticercus stage of this parasite (*Cysticercus fasciolaris*) is directly associated with hepatic sarcoma in 90 per cent of the cases, acting, however, merely as a chronic irritant.

There is no evidence in our observations to support the assumption that substances derived from the dead parasite are more efficacious in the production of tumors than the excretions of the living *Cysticercus fasciolaris*.

Epithelial tumors are much more frequent in rats than is generally supposed.

Since this paper went to press and up to December 20, 1916, sixteen additional spontaneous tumors developed in the laboratory stock. These were classified as follows:

Adenofibroma: breast	5
Sarcoma: liver	6
Sarcoma: perineum	1
Carcinoma: kidney	2
Epithelioma: head	1
Myeloma: lymph glands	1

All of the sarcomata of the liver originated in the walls of parasitic cysts. In two of the cysts showing early sarcomatous transformation, the *Cysticercus fasciolaris* was alive, while in the remaining four animals the parasite was dead.

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A STUDY OF SOME DIAGNOSTIC REACTIONS FOR MALIGNANT TUMORS

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Under the auspices of the Huntington Fund for Cancer Research I have investigated some of the serum-diagnostic tests for malignant tumor, chiefly those of Freund and Kaminer and of von Dungern, and it is the purpose of this communication to report the results of that study.

The serological diagnosis of disease is made with the aid of reactions that are designated as "bio-chemical" reactions because of the fact that the chemical constitution of the substances taking part in those reactions is for the greater part unknown. The first serological test applied to the diagnosis of disease (the Widal reaction) depended upon the interaction of true antigenic substances with their specific antibodies, in the phenomenon of agglutination. As other expressions of specific immunity reactions were discovered (specific complement deviation, meiostagmine reaction, anaphylactic shock) attempts were made to use these also in the diagnosis of disease, and as a result of such efforts a perfect imitation of specific complement deviation was discovered in the non-specific Wassermann reaction, a reaction that is produced by the interaction of lipoid substances lacking true antigenic properties, and a serum constituent that is not a true antibody.

In the search for a diagnostic serological test for malignant tumors, the investigations have generally followed the lead of the specific as well as of the non-specific immunity reactions, but some tests have been devised that are not based on any of these reactions.

The experimental basis of the search for a specific immunity reaction in cancer is found chiefly in the studies upon immunization against normal tissues. These have demonstrated antibody production against tissues derived from foreign species, against the cells of animals of the same species (1) and against the cells of certain of the individual's own organs (2) (kidney, pancreas and spleen). Ample ground seemed to be furnished by the results just cited for the assumption that the immunity often observed against the inoculation of homogenic transplantable tumors was due to the influence of cytotoxic antibodies, yet such antibodies have never been found. Moreover, the methods of specific precipitation and complement fixation, also, have generally (3) failed to reveal the development of antibodies in animals bearing true transplantable tumors.

In human cancer, specific antibodies have been sought with the use of the method of complement fixation, the anaphylactic reaction, and the meiostagmine reaction. The method first mentioned has failed completely in a number of hands. Ludke (4), however, believed that he could show, with the serum of two cases of human carcinoma, a slight specific complement deviation, and Simon and Thomas (5), using the hemolytic system, hen corpuscles, anti-hen corpuscle—rabbit's serum and guinea pig complement, obtained complement deviation in 24 out of 37 cases of malignant tumor, by uniting the patients' sera with a quantity of cancer extract that by itself was not anticomplementary. They obtained no positive reaction in 50 cases of normal or otherwise diseased individuals.

Ranzi (6) injected 10 cc. of a paper-filtered cancer extract into two cancer patients and after one week examined the two sera with the method of complement deviation. His results were entirely negative. Similar experiments were carried out by Lebredo and Coca (7), all of which resulted negatively.

The anaphylactic reaction has been used in both its local and its general expression. von Dungern and Gorowitz (8) reported a specific local hypersensitiveness in cancerous individuals to extracts of malignant tumors. Their observations have not been confirmed. Pfeiffer and Finsterer (9) employed the method of passive sensitization to demonstrate specific immune bodies in human individuals suffering from cancer. Three guinea pigs received intraperitoneal injections of 4 cc. of the serum of human cancer patients. On the following day, each animal received intraperitoneal injections of 4 cc. of the expressed juice of human cancer. All three of these animals exhibited symptoms of hypersensitiveness from which they recovered. Five control animals, that previously had been treated with normal human serum, and two others not previously injected, showed no symptoms upon receiving similar inoculations of the cancer juice. Ranzi (10), in a series of nine cancer cases and fifteen controls, failed entirely to confirm the results of Pfeiffer and Finsterer. Ranzi's test injection was made intravenously and not intraperitoneally, as prescribed by Pfeiffer and Finsterer. Philosophow (11), in two cases, also, was unable to confirm the experiments of Pfeiffer and Finsterer. Later, Pfeiffer (12) published a larger series of experiments by which he upholds the results obtained with Finsterer. According to these experiments, tumor-specific antibodies are demonstrable in the blood of individuals suffering from carcinoma but not of those afflicted with sarcoma or non-malignant tumors. The usual criteria of hypersensitiveness—convulsions or death—were not used by Pfeiffer and Finsterer; they employed the single criterion of temperature drop. This phenomenon was first employed by Pfeiffer (13) in the detection of the lesser degrees of anaphylactic reaction, and it was later claimed by Mita (14) that with the use of a certain formula the degree of shock could be exactly calculated by noting the temperature curve over a period of several hours after the toxic injection.

The writer was discouraged from undertaking a further study of the Pfeiffer reaction in cancer on account of some unpublished experiences of Dr. Richard Weil and himself. It was found, first, that the rectal temperature of normal guinea-pigs varies greatly according to the depth to which the thermometer is inserted; and, secondly, that in many animals a marked lowering of the rectal temperature is caused by merely strapping the animal on the operation board.

In the class of non-specific reactions are to be placed the Freund-Kaminer reaction, and probably also, the complement fixation test of von Dungern, notwithstanding the fact that the latter reaction is carried out according to principles drawn originally from the interaction of specifically related bodies.

The Freund-Kaminer test for malignant tumor is based upon the observation of the authors that the isolated cells of carcinoma are dissolved by the serum of non-cancerous individuals, whereas this property is wanting in the sera of the cancer patients. The technic of the test is as follows:

Preparation of the cancer cell emulsion

The non-necrotic portions of metastatic liver carcinoma are cut into small cubes and allowed to stand in the ice-box over night in 0.6 per cent NaCl solution containing 1 per cent of acid sodium phosphate. The tissue is then crushed by hand in a thin, fine-meshed towel under the acid sodium phosphate mixture, in such a manner that the isolated cells and small groups of cells are forced through the towel into the fluid outside. The cells are now washed ten times with 0.6 per cent saline solution with the use of the centrifuge (at slow speed for two or three minutes) to remove the liver cell débris, and at the end of this process the suspension is centrifuged for one-quarter minute at lowest speed, and the sediment, containing the larger tissue fragments and groups of cells that have not been separated, is discarded. The final suspension should contain only isolated cells and no cell fragments; the preparation of the cell emulsion is conducted without any precautions directed to the exclusion of bacteria. The cells in the resulting suspension are counted, and the suspension is diluted to a point at which 1 drop in 20 will give the desired number of cells per field of 16 small squares in the Thoma-Zeiss blood-counting chamber. This number has been placed by Freund and Kaminer at between 15 and 25. Finally, the cell suspension is mixed with a tenth volume of a concentrated (5 per cent or 10 per cent) solution of sodium fluoride. According to Freund the fluoride solution should be slightly alkaline to litmus. The cell suspension is kept in the ice-box.

The test

Twenty drops (about 1 cc.) of the freshly obtained patient's serum are mixed in a clean narrow glass tube with one drop of the (shaken) cell suspension and two drops of the concentrated sodium fluoride solution. The number of cells per 16 small squares of the Thoma-Zeiss blood-counting chamber is determined in a drop of the mixture, and the tube, after being tightly closed with a stopper, is placed in the incubator at 37°C. for twenty-four hours. A second count of the cells is then made in order to see whether the number of cells has diminished. It is not required that the glass tubes or pipettes be sterile.

With ether Freund and Kaminer were able to extract, from normal sera, fatty acids which destroyed the isolated cancer cells. As these acids, according to Freund and Kaminer, are lacking in the serum of cancer patients, they are believed to be the active carcinolytic substances in normal sera.

The cell dissolving property of normal serum is destroyed by heat (56°C. for one-half hour). It may be preserved in serum kept in the ice box for about one week, but it is lost within two days at room temperature.

Cancer sera possess the power of inhibiting the carcinolytic property of normal sera. This inhibiting power is believed by Freund and Kaminer to be exercised by the nucleo-euglobulins which, in cancer serum, possess chemical characters different from those of the nucleo-euglobulins of normal serum. This difference is particularly indicated by a different result with the Molisch carbohydrate reaction.

The observations of Freund and Kaminer were confirmed by Ranzi and Amiradzibi (15), by Arzt (16), by Stammler (17), by Kraus and Graff (18), who found further that the blood of the umbilical cord behaves toward the cancer cells as does the blood of cancer patients, in that it lacks carcinolytic properties; by Kraus, von Graff, and Ranzi (19), by von Monakow (20), who, however, observed over 50 per cent destruction of the cancer cells with only 16 out of 52 normal sera; and by Arzt and Kerl (21).

My own experience in the Freund-Kaminer phenomenon began in the chemical laboratory of the Rudolf-Stiftung under the direction of Professor Freund. There were placed at my disposal 0.6 per cent sodium chlorid solution (not sterile) 10 per cent[1] sodium fluorid solution (Merck's Fluornatrium puriss) and eight different emulsions of the cells of secondary carcinoma of the liver. Examination of these cell emulsions showed three of them to contain cells of uniform size with almost no fragments, the presence of which might disturb the counting of the cells. These three emulsions were used in the subsequent experiments.

The cell emulsions were of such a density that when one or two drops were mixed with 1 cc. (about 20 drops) of serum and two drops of the sodium fluorid solution, and a small drop of the mixture was placed in the Thoma-Zeiss blood counting chamber, the average[2] number of cells in a field of 16 small squares lay usually between 15 and 25.

The first experiment was carried out with fresh serum from a case of carcinoma of the breast and from a case of advanced arteriosclerosis (non-cancerous), each of which was tested with two of the cell emulsions. The results were as follows:

Cell emulsion A

Cancer serum	At once	9, 14, 5, 11, 11, 16, 11, 12, 14, 7	(av. 11)
	After 24 hours	11, 11, 9, 10, 7, 10, 15, 5	(av. 10)
Arteriosclerosis serum	At once	15, 13, 12, 9, 16, 14,	(av. 13)
	After 24 hours	8, 5, 4, 5, 6, 5, 5	(av. 5.5)

Cell emuslion B

Cancer serum	At once	22, 20, 25, 22, 21	(av. 22)
	After 24 hours	23, 20, 27, 23, 23	(av. 23)
Arteriosclerosis serum	At once	18, 23, 29, 22, 24	(av. 23)
	After 24 hours	6, 5, 4, 9, 4	(av. 5.6)

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[1] The 10 per cent solution of sodium fluoride was prepared according to Freund and Kaminer by shaking 10 gr. of the substance with 100 cc. of water and filtering the mixture. The filtrate did not contain 10 per cent nor even 5 per cent of sodium fluoride, since the saturated aqueous solution of this substance contains less than 5 per cent of it by weight. In fact, the solids in the filtrate were found, by weighing the dry residue from 1.0 cc. of the filtrate, to amount to 4.5 per cent, which shows the solution to have been not quite saturated.

[2] Between 5 and 10 fields were counted and the average of these was taken.

It is seen that, when mixed with the non-cancerous serum, the cells of both emulsions were markedly diminished in number after twenty-four hours at 37°C., whereas the cancer serum left the number of cells, in both instances, practically unaltered.

The next series of tests was carried out with the object of studying the nature of the cytolytic agent in normal sera. For these experiments, fresh normal human sera and horse serum were used, the other reagents being the same as in the first experiment. However, in these tests no cytolysis took place.

Bacteriological examination was made of the mixtures of this series before and after the twenty-four hour incubation, for the purpose of determining the antiseptic activity of the sodium fluorid. This examination was carried out by spreading one loop of each of the mixtures on the surface of nutrient agar and counting the number of colonies that had appeared at the end of forty-eight hours. The cultures taken before incubation showed either no growth or at most two colonies, whereas the cultures from all the mixtures taken after the twenty-four hours' incubation at 37°C. showed numerous colonies within twenty-four hours after the cultures were taken. The number of colonies that developed from the mixtures containing the sodium fluorid was, however, considerably less than those that developed from the mixtures in which sodium chlorid solution (0.6 per cent) was substituted for the sodium fluorid. This result indicates that the antiseptic action of the fluorid, as it is used by Freund and Kaminer, is slight. After numerous failures to obtain cytolysis with normal human sera, this phenomenon was again observed with a normal human serum and with normal horse serum. The human serum referred to was tested with two other normal sera and one cancer serum. In the mixtures with the latter three sera, the cancer cells were clumped and could not, therefore, be counted, but according to the gross appearance of the sediment as compared with the sediment in the salt solution control (containing no serum), only partial solution of the cells occurred with one of the other normal sera, while with the third normal serum and with the cancer serum no cytolysis could be detected. The first mentioned normal human serum in this series caused almost complete solution of the cancer cells, the count being:

At once	19, 22, 21, 23, 12, 27, 16 (av. 20)
After 24 hours 37° C	0, 0, 0, 1, 0, 0, 1

Bacteriological cultures from all the mixtures taken after the twenty-four hours' incubation period, showed countless fine white colonies. This result indicates that the cytolysis is neither hindered nor produced by bacterial growth.

The horse serum just referred to was tested at the same time with the sera of two normal rabbits, neither of which possessed any cytolytic power. The horse serum dissolved about 70 per cent of the cells of two different emulsions. In a single experiment with this horse serum, an attempt was made to examine the mechanism of the cancer cell cytolysis. The usual mixture was prepared and allowed to stand at room temperature for three hours, at which time no grossly apparent cytolysis had taken place. The mixture was then centrifuged, and the sediment was mixed with 1 cc. of 0.8 per cent sodium chlorid solution and two drops of the saturated sodium fluorid solution (mixture A), while the decanted supernatant fluid was mixed with two drops of the cancer cell suspension (mixture B). The cells in both of these mixtures were counted before and after the incubation at 37°C. for twenty-four hours, with the following results:

Mixture A	At once	22, 22, 14, 26, 21, 25, 22 (av. 20)
	After 24 hours	24, 21, 18, 11, 8, 14, 14, 14 (av. 14)
	II	
Mixture B	At once	24, 21, 18, 18, 23, 17 (av. 20)
	After 24 hours	16, 15, 12, 25, 16, 12, 27 (av. 17)
Control usual mixture	At once	16, 14, 18, 21, 25, 17, 25 (av. 19)
	After 24 hours	3, 4, 4, 7, 4, 4, 9 (av. 5)

The distinct diminution (30 per cent) in the number of cells in mixture A indicates that the cells at the end of three hours, though still apparently intact, had already combined with some of the cytolytic agent in the serum. It is possible that most of the agent had been absorbed by the cells but was inhibited in its action by unfavorable conditions of the saline menstruum.

At any rate, little, if any of the cytolytic activity remained in the serum after its three-hour contact with the cells.

Further study in this direction was prevented by the fact that in none of the numerous subsequent tests has any cytolytic activity been observed in any fresh normal human or other serum.

The subsequent experiments were carried out in the cancer institute of the Eppendorferkrankenhaus in Hamburg, in the General Memorial Hospital in New York City, and in the New York Hospital. The cell suspensions used in Hamburg were prepared, according to the Freund-Kaminer prescription, from two metastatic carcinomas of the liver that were obtained from the pathological institute. Only non-cancerous sera were used, these being derived largely from syphilitics and individuals suffering from tuberculosis and other diseases. The same sera were tested, also, with a cancer cell suspension brought in a thermos bottle from Vienna. The cell suspensions used in the General Memorial Hospital were prepared from a sarcoma and a cellular mammary carcinoma, both being fresh surgical material received from Dr. W. B. Coley. The sarcoma cell suspension could not be used, as the cells soon agglutinated into a gelatinous mass that could not be broken up by shaking.

A suspension of cells from a sarcoma of the testicle, obtained from operation at the New York Hospital, remained in suspension in the saline solution but became agglutinated when mixed with human serum. The other sources of cell material used at the New York Hospital were two metastatic liver carcinomas, one of which was received from the General Memorial Hospital through Dr. Ewing.

All of the more than 150 experiments in the three institutions resulted negatively, no cytolysis being observed in any instance with fresh normal human, dog, or horse serum.

It is evident from these experiences with the Freund-Kaminer phenomenon, that the cytolytic action of normal sera is dependent upon some factor as yet uncontrollable, and that that action, therefore, can not be made the basis of a differential test for malignant tumor.

The technic of von Dungern's complement-fixation test for malignant neoplasms (22) has been modified in several respects by von Dungern since its first announcement.

The antigen extract consisted at first of an alcoholic extract of malignant tumors. For this preparation von Dungern substituted, in his second report, an acetone extract of human blood corpuscles, particularly those of individuals suffering from progressive paralysis. Hara (25) later used maltose and phenolphthalein as "antigen" in the test.

In the first experiments, the patient's serum was used unheated in a quantity of 0.05 cc. In his second report, von Dungern still used the same quantity of unheated serum, either as usual or with the addition of 0.2 cc. of n/50 NaOH. In his third publication, von Dungern recommended that the serum be heated for one-half hour at 54°C., after having been mixed with two parts of n/50 NaOH in about 0.6 per cent NaCl. Of this heated mixture, he used in series 0.6, 0.3, 0.15, and 0.075 cc. However, the tests were judged by the results with one quantity, 0.3 cc. of the mixture.

Von Dungern requires the use of sensitized ox blood corpuscles instead of the customary sheep's corpuscles, as the indicator of the cancer reaction, on the ground that they are less sensitive than the sheep's corpuscles in the desired capacity. Another departure from the usual Wassermann technic is the three hour fixation period at room temperature and the three hour incubation period for hemolysis, also at room temperature.

Petridis (24), working in von Dungern's laboratory, used the acetone extract of human blood corpuscles as "antigen," and followed the third method of von Dungern in the use of the patient's serum. Since the article of Petridis (24) there has been no published modification of the technic of the test, excepting, as has been said, the substitution, by Hara, of maltose and phenolphthalein for the "antigen" extract.

With the final technical form of the test von Dungern obtained nearly 91 per cent of positive reactions in malignant tumor, and 100 per cent of negative reactions in non-cancerous conditions, including syphilis. Halpern (25), in von Dungern's laboratory, obtained 89.8 per cent of positive reactions in malignant tumors, with 92.8 per cent of negative reactions in non-cancerous conditions. Petridis (24) obtained 81.2 per cent of positive reactions in malignant tumors, and 84.2 per cent of negative reactions in non-cancerous conditions. Hara (26) reported about 84 per cent of positive reactions in malignant tumors, and over 97 per cent of negative reactions in non-cancerous conditions. Wolfsohn (27) obtained only 80 per cent of positive reactions in malignant tumors, and only 63 per cent of negative reactions in non-cancerous conditions. Wolfsohn found, further, that many luetic sera reacted positively with the cancer test as prescribed by von Dungern. A similar experience was reported by Edzard (28), who obtained only 70 per cent of positive reactions in malignant tumors, and only 65 per cent of negative reactions in non-cancerous individuals. Lindenschatt, on the contrary, obtained no positive cancer reactions with luetic sera.

Isabolinsky and Dichno (29) report unfavorable results with the test, and H. Sachs[3] failed to confirm von Dungern's observations.

My first experiments with the cancer reaction of von Dungern were carried out in the Loomis Laboratory, with an acetone extract of normal human corpuscles and with sera of cancer patients obtained through the kindness of Dr. Richard Weil from the General Memorial Hospital. Thirty-six cancer sera and an equal number of non-cancerous sera were tested, and in no case was a fixation of complement obtained that could not be explained either as a summation effect, on account of the fact that the serum control, in which double the test amount of the serum alkali mixture was used, often caused a complete inhibition[4] of the complementary action, or as an expression of a Wassermann reaction, which in a few instances was positive with the usual technic.

These experiments were continued in Hamburg in von Dungern's institute. There a second considerable series of cancerous and non-cancerous sera were tested with the blood extract then in use in the routine examinations of the institute, and also with several other extracts of normal and syphilitic blood corpuscles. Here, again, my tests with the prescribed technic resulted negatively in every instance but one. Furthermore, all the routine tests of the institute that I saw during my stay in Hamburg resulted negatively, although many of these, also, were carried out with sera from cases of known malignant disease.

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[3] Personal communication.

[4] Sometimes even the amount of the serum-alkali mixture used in the test was by itself slightly anticomplementary.

On two occasions, however, in which the prescribed technic was modified I obtained a clearly positive result, and in one of these instances the reaction appeared to be specific in a clinical sense. In the first instance, a single cancer serum and one Wassermann positive leutic serum were tested with the usual blood corpuscle extract and also with the lipoid (acetone insoluble) fraction of the same extract. The technic with both of these "antigen" preparations was that of the routine institute tests, and with both of the preparations the cancer serum reacted positively, whereas the luetic serum reacted negatively. A further series of seven cancer sera were tested according to von Dungern with the isolated human and pig's blood lipoids, the results being in every case negative.

In the second instance referred to above, the tests were performed, not with the serum but with the urine and with a solution of the alcohol precipitate of the urine of two individuals, one of whom was suffering from carcinoma, the serum of the other being strongly positive with the Wassermann test.

The patient's serum substitute was prepared as follows: 100 cc. of urine were mixed with 400 cc. of 96 per cent alcohol and the precipitate, after being washed once with 96 per cent alcohol, was taken up with distilled water up to 20 cc. The small part of the precipitate that remained was diluted one-fifth with physiological saline, and 0.025 cc. of this dilution was used in the tests in place of the patient's serum.

The urine in both cases was negative both to the usual tests for albumin and to the biuret reaction; the cancer urine was alkaline, the other was acid. In the concentrated solution of the alcohol-precipitate from the cancer urine, urobilin could be demonstrated spectroscopically, and its presence was further indicated by a positive biuret reaction in the concentrated solution. The solution of the alcohol-precipitate from the non-cancerous (Wassermann positive) urine contained no demonstrable urobilin.

TABLE 1

	ACETONE EXTRACT OF GUINEA PIG'S HEART 1 PER CENT IN METHYL ALCOHOL				ACETONE EXTRACT OF WASHED HUMAN BLOOD CORPUSCLES (CASE OF PROGRESSIVE PARALYSIS) 1 PER CENT IN METHYL ALCOHOL			
	Cubic centimeters				Cubic centimeters			
	0.05	0.025	0.0125	0.00625	0.05	0.025	0.0125	0.00625
Kittelberger (carcinoma) urine, 0.025 cc.	+	−	−	−	++	++	++	−
Alcoholic precipitate solution, 0.025 cc.	+	−	−	−	++	++	+	−
Same boiled, 0.025 cc.	++	++	+	−	++	−	−	−
Schmitt (*Wassermann positive*) urine, 0.025 cc.	++	++	+	−	−	−	−	−
Alcoholic precipitate solution, 0.025 cc.	++	++	−	−	−	−	−	−
Same boiled.	++	++	+	−	+	−	−	−

+ = degree of fixation of complement.
− = no fixation of complement.

It is seen that the cancer urine (Kittelberger) reacted positively with the blood extract (von Dungern) and negatively with the guinea pig's heart extract (Wassermann). On the other hand, the control urine (Schmidt) reacted positively with the heart extract and negatively with the blood extract, an identical differential result was obtained with the solution of the alcoholic precipitate, and furthermore, it was found that after the solution of the cancer urine alcohol sediment had been boiled, its reactivity with the Wassermann and von Dungern extracts was reversed.

A further series of 98 sera from cases of carcinoma and sarcoma were tested in the General Memorial Hospital according to von Dungern and according to Wassermann; 13 of these were Wassermann positive, and, of the 13, four caused complement fixation also with the blood extract, which was prepared from washed blood corpuscles from a case of tertiary syphilis. In no other case was complement fixation observed that could be interpreted as a reaction of diagnostic significance.

These experiences with the complement-fixation test for cancer point to the possibility that the positive results that have been reported are due in part to accident and in part to summation effect, or, as Sachs suggests, that a hitherto unknown and uncontrolled factor is required for the successful application of the method.

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EPITHELIOMA DEVELOPING IN A SKIN ULCER IN PELLAGRA

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The origin of squamous epithelioma in the skin ulcer of pellagra must be an extremely rare occurrence. I have been unable to find a record of any previous case. A brief report of this case may therefore be of interest.

The patient in whom it occurred was a negro woman, twenty-two years of age, who entered the Roper Hospital on February 15, 1916, suffering from acute pellagra and died on February 17, 1916. She said she had been well until two months ago and that ulceration had been present on the inner surface of the thighs for four weeks.

The rough, scaly, pigmented pellagrous dermatitis was present over the dorsal surface of both hands, the elbows, and the extensor surface of the legs. The epiderm over the left elbow was sloughing, leaving a moist ulcerated surface. On the inner surface of the thighs were symmetrical superficial ulcers about the size of the palm of the hand, with red, smooth, moist base continuous with the skin at the edge.

At the autopsy a section for microscopic study was taken from the edge of one of these denuded areas on the thigh as a routine measure.

Microscopically the epithelial layer away from the ulcer is thick, but it gets thin and is lost in the ulcer. There is considerable degeneration of the outer layers of cells where it is present. The whole of the section shows an extensive growth of cords of epithelium with numerous young pearly bodies and cancer bodies infiltrating the subepidermal tissues beneath the

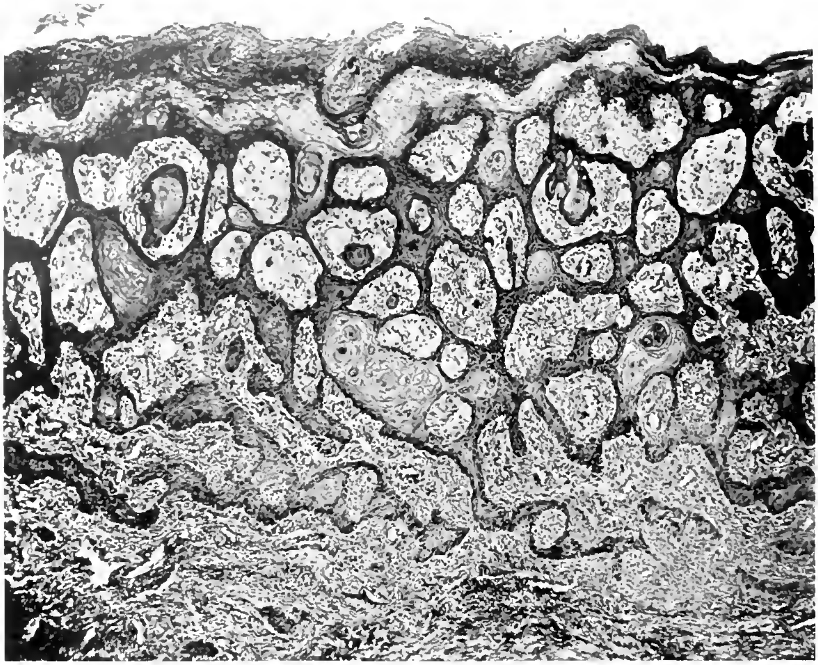

FIG. 1. Squamous epithelioma developing in pellagrous skin ulcer. Spencer 16 mm. obj., 10 × oc.

ulcerated surface and also beneath the epiderm at its edge. These cords originate at numerous points from the base of the epithelium. No surface growth is noted. In the lower parts of the section particularly there is marked lymphocytosis, connective tissue growth and new blood vessel formation.

None of the regional lymph glands was taken and no kindred condition was found in the other parts of the body.

THE IDENTIFICATION OF THE CELLS IN MYELOMAS BY MEANS OF THE INDOL-PHENOL BLUE SYNTHESIS

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The type of the tumor cell in myelomas is still in doubt. By some it has been classified as a myeloblast (MacCallum (6)), and by others its origin has been assigned to another cell, which is described by them as a bone-marrow plasma cell. In certain cases its identity with the erythroblast has been established (Ribbert). Schridde in Aschoff's Pathologische Anatomie mentions "eine eigene Beobachtung, bei der die dunkelroten Geschwulstmassen sich aus Myeloblasten und zurücktretenden Erythroblasten zusammensetzen (Erythro-Myeloblastom)." Mallory (7) says of it, "Evidently it does not belong to the myeloblast series of cells because it does not differentiate like them. Moreover, the myeloma is never associated with myelogenous leukemia." Since it has been definitely established that the oxydase reaction is characteristic of myeloid cells, the application of this reaction to the cells of a myeloma ought to be of great service in identifying these tumors.

While experimenting with this reaction along other lines, an opportunity of applying it to a specimen of myeloma presented itself. This paper embodies the results of this work. This is in keeping with the suggestion of MacCallum in his text book of pathology, that the reaction is to be recommended as a future diagnostic method because the cells, at least, those of the myeloblastic type, ought to contain oxydase ferments.

The indol-phenol blue synthesis of Ehrlich, or oxydase reaction, was applied by Winkler in 1907 to pus smears and to sections of tissue for the identification of the granules of oxydase ferment. Schultze after extensive application of this reaction to the various tissues of the body found that, after formalin fixation, only cells from the lachrymal and the thyroid glands, together with the cells of the myeloid group, gave this reaction. More recently in this country, F. A. Evans has applied the reaction to the identification of leukocytes in the circulating blood (1) in exudations (4) and in the tissues (5). The careful review of the literature by Evans makes it unnecessary to give in detail the historical side of this subject.

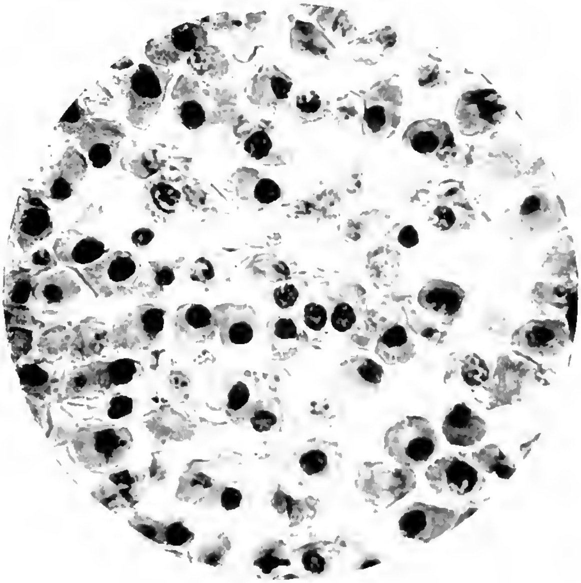
MATERIAL

The specimen upon which this study is based consists of a tumor involving the lumbar vertebrae. In its growth it has replaced the bodies of these vertebrae, leaving only a shell of bone. The blood examination made upon the patient about two weeks before his death, shows that there was not a leukemic state present in the blood at that time.

The specimen has been preserved in neutral formalin, while sections of the tissue which had been placed in Zenker-formol at the time of the autopsy have been cut at four microns in paraffin. These have been stained with a variety of stains including hematoxylin and eosin, eosin and methylene blue, phosphotungstic acid hematoxylin, iron alum hematoxylin, Mallory's aniline blue for connective tissue, aniline acid fuchsin methyl green.

The tumor cells vary considerably in size. Their cell body is irregular in shape, as a rule compressed, and with an indefinite boundary. After formalin fixation, under an oil immersion lens, the unstained cytoplasm of the typical tumor cell shows definite granules. After Zenker-formol fixation the cytoplasm is as a rule neutrophilic, although some of the cells present an eosinophilic cytoplasm. A certain number of these cells, moreover, present definite eosinophilic granules and are indistinguishable from the eosinophilic myelocytes of the normal bone marrow. The nucleus of many of the tumor cells is eccentrically placed. Its coarse chromatin granules are grouped about the periphery so that some of these cells come to resemble quite closely the plasma cell. With the eosin-methylene blue and anilin acid fuchsin staining methods after Zenker-formol fixation, the nucleolus is strikingly prominent, being stained a bright red. Mitotic figures are numerous.

A few large multinucleated cells are seen in every section studied. None of these are undergoing mitosis, but the nuclei are separate and distinct. There are also many cells which present a small pyknotic nucleus and cytoplasm which is apparently hemoglobiniferous. It is intensely eosinophilic and fuchsinophilic, and stains blue with phosphotungstic acid hemotoxylin. There are also numerous red blood cells seen intermingled with the tumor cells. It is, therefore, possible that we are dealing here with a tumor similar to the one mentioned by Schridde as an erythro-myeloblastoma.



A Typical Area from the Tumor

Stained with hematoxylin and eosin. 1.8 mm. Spencer objective, ocular 8

An occasional cell contains vacuoles which we believe to be fat, since the sections prepared with fat stains present droplets of similar size and of about an equal number and distribution (vide infra).

The cells are closely arranged in a delicate reticulum, containing large and poorly formed blood-vessels. The presence of cells which resemble to some extent, at least, the plasma cell together with those appearing as if they were myeloblasts makes this an excellent specimen for the application of the oxydase reaction.

TECHNIQUE

The indol-phenol blue synthesis depends upon the fact that alpha naphthol and dimethylparaphenylendiamin combined with two atoms of oxygen forms indol-phenol blue.

Formalin appears to be an essential part of every fixative which has been successfully employed. We, however, are unable to obtain the reaction in paraffin sections after Zenker-formol fixation. The study, therefore, has been confined to tissue fixed in 10 per cent formalin which has been neutralized over magnesium carbonate. This tissue has remained in the formalin solution for about three weeks, the reaction, however, can be obtained in tissues preserved in formalin for over a year, according to Evans ('15b).

Frozen sections were prepared and submitted to the following technique:

1. Equal parts of freshly prepared solutions of 1 per cent alpha naphthol in 1 per cent KOH and of 1 per cent dimethylparaphenylendiamin (Merck) are mixed and placed on the section for two minutes.
2. The section is washed with distilled water, mounted in water, and examined with a 1.8 mm. objective.

These sections can be counterstained with an aqueous solution of safranin. We, however, found that this is not satisfactory as the counterstain diffuses into the mounting medium. By crushing the section after it is mounted, it is possible to study the isolated cells. The character of their nuclei can in this way be so easily identified that a counter stain is not at all necessary. Attempts to make permanent mounts proved so unsatisfactory that they were abandoned and only water was employed as a mounting medium. These preparations can be used for an hour to an hour and a half, giving sufficient time for study.

DISCUSSION

A very large majority of the tumor cells give the oxydase reaction. These oxydase granules are observed in the cells with an eccentrically placed nucleus, which when stained with the ordinary stains resemble plasma cells. They are found in most of the cells which resemble myeloblasts. In many of the large multi nucleated cells, it is possible to demonstrate the presence of oxydase granules. Fortunately, an occasional polymorphonuclear leucocyte is found in these sections and can be used as a positive control for the reaction. These leucocytes present the characteristic number of granules which many workers have already described.

Certain of the cells present large droplets which may be confused with the true oxydase granules. The former, however, stain a red violet color, indicative of fat, and are easily distinguished from the blue black granules of indol-phenol blue. Sections stained with Herxheimer's solution present about an equal number of cells containing similarly sized droplets of fat.

Since the application of the indol-phenol blue synthesis to tissues has been established as a criterion for the differentiation of the cells of the myeloid group, it proves itself of great value in the identification of the cell types seen in myelomas. The cells in the specimen studied by us with this oxydase reaction are definitely placed in the myeloid group.

The absence of the oxydase granules in a few of these cells affords grounds for interesting speculation. It has been suggested that immature myeloid cells might not contain them. Since, however, the tumor is composed of rapidly proliferating and undifferentiated cells and since the majority of these cells do contain oxydase granules, the elaboration of an oxydase ferment must occur very early in the life history of these cells.

The fact that these granules are present in many of the cells, which by their shape, size, and staining reaction somewhat closely resemble the plasma cell, leads us to believe that many of the specimens which have been described as plasma cell tumors are composed of cells of myeloid origin.

The more intensive study of myelomas and related conditions by the application of the indol-phenol blue synthesis should be of great aid in determining the histogenesis of these growths.

SUMMARY

1. The application of the indol-phenol blue synthesis is widely recognized as a differential characteristic of cells of the myeloid group.

2. This reaction has a practical significance in the identification of the cells seen in myelomas.

3. The tumor cell in the specimen studied by its morphology and behavior warrants the diagnosis of myeloma.

4. The presence of the oxydase granules in the tumor cells of this specimen places them definitely in the myeloid group.

We are indebted to Dr. Ernest Scott for the material and permission to make this study, and to Dr. C. C. Hugger for the microphotograph.

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THE PALLIATIVE TREATMENT OF INOPERABLE CARCINOMA OF THE CERVIX BY MEANS OF RADIUM

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The various palliative treatments formerly employed in cases of inoperable carcinoma of the cervix have proved so unsatisfactory that any improvement should be hailed with joy by the profession. Reports of the success of radium treatment, therefore, deserve attention and investigation, since these reports have appeared in the last few years with such frequency that the method may now be considered firmly established. The technique and application, the range of usefulness, the permanency of the relief, the histological changes taking place, and the process by which the rays produce their effects are, however, questions which are still unsettled; and the purpose of this article is to contribute to the various phases of the subject not yet cleared up, and to put on record sundry interesting observations made during the treatment of a small but varied series of cases.

Under ordinary circumstances, it would be unwise to report such a series of cases at so early a stage of treatment; but as no claim is made that permanent results have been obtained, it has been thought permissible to describe the early palliative effects, without attempting to predict whether the improvement is more than temporary.

The writer has treated twelve cases of carcinoma of the cervix in the gynecological wards of Mt. Sinai Hospital, eleven on the service of Dr. Joseph Brettauer, and one on that of Dr. H. N.

Vineberg. He takes this occasion to thank Dr. Brettauer and Dr. Vineberg for putting the cases at his disposal. Two patients withdrew from treatment and were lost sight of after two radiations each, before the result could be observed. His technical experience has been enlarged by the treatment of fourteen other patients, including those suffering from vulvar, rectal, gastric, laryngeal, etc., conditions; these, however, will not be referred to, as they do not bear directly upon the subject discussed in this paper.

Radium employed. Through the kindness of Dr. Francis Carter Wood, radium bromide, equaling 130 mgm. of pure radium, was placed at the disposal of the writer. The radium was distributed in four glass tubes containing respectively 83, 20, 17, and 10 mgm. The length of the largest tube (28.7 mm.) at times made awkward its application within the vagina. In most instances, 120 mgm. were used; in some cases, only 47 mgm. were employed.

TECHNICAL

Screening. As it is essential that the soft alpha and beta rays be removed by filtration, brass or lead was interposed between the glass radium container and the tissues. These metal filters in turn generate soft secondary rays which are as destructive to the tissues as the soft primary rays, but the secondary rays are readily absorbed by para rubber, gauze, or paper.

Preparation of radium carrier. The radium was arranged differently in each case, depending upon the site of the lesion, the size of the growth, and the dimensions of the vagina.

1. If 47 mgm. only were used, the three naked glass tubes of 10, 17, and 20 mgm. were placed in a hollow lead cup attached to a handle. The opening was covered with a lid of brass, 1 to 1.5 mm. thick, which was fastened with a layer of adhesive plaster, and the cup was inclosed in a small bag of rubber, 1 mm. thick (fig. 1). The use of the lead cup was suggested to the writer by Dr. Howard A. Kelly of Baltimore, who employs it in the treatment of cervical carcinoma. The cup has the advantage that the rays are cut out in every direction but one,

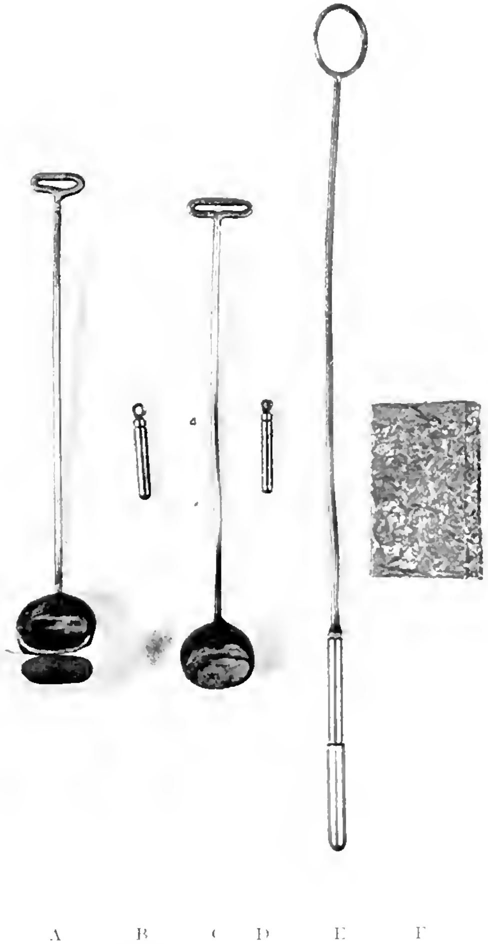

FIG. 1. INSTRUMENTARIUM USED FOR RADIUM APPLICATION TO UTERUS CERVIX AND VAGINA

A, Hollow lead cup on handle, showing brass cover attached with adhesive plaster and thrown back. Hollow used to contain the radium. *B* and *D*. Brass container for 17 and 10 mgm. capsules of radium. *C*. Thinner walled lead cup without cover. *E*. Brass containers in tandem arrangement for radiation of corpus carcinoma. *F*. Numerous layers of lead foil which are used to protect healthy parts.

and that the attending nurse is able to change the direction of the rays without disturbing the patient. It is most useful in carcinomata of small size where concentration of the rays is desired.

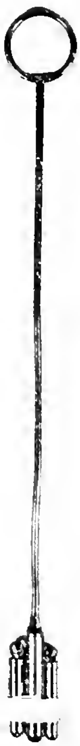

FIG. 2. TRIDENT ARRANGEMENT CONVENIENT FOR RADIATION OF LARGE GROWTHS INFILTRATING THE VAGINAL WALL

2. For large cauliflower growths, especially such as were accompanied by diffuse downward infiltration of the vaginal walls, the following arrangement was found to be most advantageous: The 83 mgm. tube was inclosed in a brass capsule, 1.5 mm.

thick, which was screwed to a handle. Around the periphery of this tube the three other tubes were placed in a similar container, but without handles. They were secured to the central tube with strips of adhesive plaster, surrounded with a rubber bag, and introduced as one (fig. 2). If, for example, the anterior vaginal wall be infiltrated, but the rectovaginal septum be unaffected, one side of the carrier may be additionally screened by four to eight thicknesses of lead foil, applied inside the rubber filter. If the lead protector be placed facing the rectum, this viscus is protected from unnecessary radiation, while the affected anterior wall receives a larger quantity of radiation.

3. In crater-like carcinomata, extending up into the cervix and involving the parametria, the arrangement was slightly varied, the long central tube being advanced 1 to 2 cm. in front of the three smaller tubes. This permitted the introduction of the projecting tube within the cervix, the smaller tubes at the same time radiating the vaginal portion and fornices (fig. 3).

Introduction of the carrier. The radium is introduced under guidance of sight, after the vaginal walls are separated with spades or retractors. Unless the carrier is to be introduced within the cervical canal, a layer of gauze about 0.5 cm. in thickness is interposed between the growth and the carrier. The carrier, fastened to the handle, is pushed firmly against the gauze, and then the vagina is tightly packed with gauze, so that its walls are separated as far as possible from the radium. The handle is secured to the vulva with strips of adhesive plaster, exactly as a permanent catheter is fixed in position. A dressing and T binder complete the operation. Patients who suffer pain are given a liberal injection of morphine to keep them quiet and comfortable.

Length of application. It is rarely possible to introduce the radium for more than eighteen to twenty-four hours at one session, because the patient must remain quiet during the treatment. An application of 120 mgm. for twenty-four hours gave the large dosage of 2880 mgmhrs. (milligram hours). A considerable part of the radiation, however, is absorbed by the 1.5 mm. of brass or 0.5 to 2 mm. of lead, the 1 to 3 mm. of rubber, and the gauze, all of which are interposed between the radium and the tissues.

Frequency of application. The frequency of application depends largely upon the local conditions, the amount of reaction (pain, rectal and bladder tenesmus, change in body weight, symptoms of absorption or toxemia), etc. In general, the following method has proved most satisfactory to the writer, but individual preference and experience will suggest wide

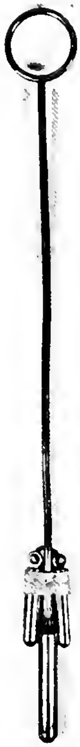

Fig. 3. Central Applicator Advanced to Permit Introduction Within Vaginal Canal, the Latter Containers Radiating the Portio

variations. The further advanced the growth, the larger the initial dose indicated. The second treatment should be given seven to ten days after the first; the third treatment, fourteen days after the second. If improvement becomes apparent (cessation of pain, hemorrhage, and foul discharge, shrinkage and cleansing of the cancer surface), the next two treatments should be given at intervals of three weeks in diminishing dosage. For the first series 5000 to 6000 mgmhrs. are sufficient; and, thereafter, one treatment each month for two or more months completes the primary treatment. The patient should present herself for examination at least once in four weeks, in order that general appearance, general health, local conditions, and weight, may be observed. Should recurrence manifest itself, a new series of radiations may be undertaken.

CLINICAL

Local effects of radiation. In every case so far treated a distinct amelioration of pain was observed within two weeks after the second radiation. Coincidentally, the bleeding diminished or disappeared, and the foul discharge become odorless and serous in character. In the earlier cases treated, because of insufficient filtration, very annoying rectal and vesical tenesmus was complained of, usually most marked on the third and tenth day after the beginning of the treatment. Severer rectal tenesmus developed in these cases as late as the third month. Since the practice of distending the vagina with gauze has been adopted, these unpleasant symptoms have either not appeared or have been very mild in character.

Between the third and fifth week after the beginning of the treatment, the cervix was observed to contract and shrink, assuming a characteristic senile size and conformation. The interior of the cervix and, sometimes, the vaginal portion were covered with a densely adherent, thick, yellow white, "bacon-like" exudate, which persisted for months. The thickening of the parametria and vaginal infiltration (much of which is usually of an inflammatory nature) gradually diminished. At a later date (after three to six months) the parametria again became more rigid (scar tissue?).

General effects of radiation. With the disappearance of the pain, bleeding, and discharge, the patient's general condition rapidly improved. In several instances (large, sloughy growths), a mild toxic condition, not marked by rise of temperature and transient in duration, was observed. In most cases, a rapid increase in weight was noted. Several of the patients appear to enjoy perfect health at present; in several others, a progressive loss of weight and strength and undefined pains and malaise are noted after a period of several months of well-being. In one of these, an enlarged nodular liver signifies hepatic metastases; in two others, in spite of the deterioration of health, no physical signs as yet denote recurrence.

Applicability of the treatment. (a) To advanced cases. Up to date, no case has been refused treatment. As will be noted from the histories, two cases had been given up as hopeless, after repeated local cauterizations (thermocautery) had proved even temporarily ineffective. One patient had had two operations (hysterectomy and secondary operation for recurrence) followed by local recurrence in the vaginal vault. In several instances, the recto- and vesicovaginal septa were involved. One patient with fracture of the humerus (x-ray diagnosis: "pathological fracture of the humerus, suspicious of metastatic involvement") is now under treatment.

The writer sees no contraindication to the treatment of even the most advanced cases. Even if no permanent relief is afforded, prompt cessation of pain, bleeding, and discharge can be obtained. A more merciful death from internal metastases or cachexia may then be anticipated. Only where carcinomatous perforation into the bladder or rectum is imminent, will radiation, by hastening the disintegration of the carcinomatous tissue, favor cloaca formation.

(b) To early cases. The operable cases of carcinoma of the cervix which have come under the writer's observation during the last ten years have been few in number (23). The final results of the radical operation, except in the hands of a few operators who control a large material, have been discouraging.

In the light of the experience of others and of the two cases reported below (cases 4 and 5), the writer feels inclined to advise, at least tentatively, that operable cases be subjected to a short preliminary treatment by radiation, followed by an abdominal total hysterectomy and salpingo-oophorectomy without excision of the parametria. It is, however, not justifiable, in the present state of our knowledge, to rely solely upon radium treatment in operable cases, unless the patient is an exceptionally poor risk (excessive obesity, severe cardiac, pulmonary, or renal disease), because there is as yet not sufficient evidence that a permanent cure can be produced by radium.

The preliminary radium treatment should be vigorous and short in duration. "Sterilization" of the growth should be sought, but the operation should be undertaken before the parametrial scar tissue formation, which regularly follows radiation, has had time to advance too far. In case 4, seven weeks elapsed between the beginning of treatment and the hysterectomy. The pelvic connective tissues were found to be so hard, contracted, and board-like, that the ureters could not be identified or pushed away from the uterine arteries. The operation consequently proved most difficult, and a transient ureterovaginal fistula subsequently developed.

Case 5 was, therefore, operated upon nine days after the beginning of radiation, and no such technical difficulties were encountered. On the other hand, microscopical examination of the carcinoma showed very few cytological changes, although the normal appearance of the cells did not necessarily exclude inhibition of cell division, the checking of division being the mode of action of radium on cells (fig. 11).

In view of these observations, the writer is inclined to advise preliminary radiation extending over a period of three weeks (three exposures of about 2000, 1200, and 800 mgm. hours each) followed within two to three days by hysterectomy. It appears inadvisable to operate before the lapse of three weeks, because the growth has not been sufficiently cleansed from a bacteriological point of view. Four weeks after operation, radium treatment should be resumed (at least three treatments at four weeks intervals).

HISTOLOGICAL

In every case a specimen was excised from the growth before the treatment was inaugurated. Usually a small specimen was removed before each subsequent radiation so that the cytological process could be followed from beginning to end. Toward the close of the treatments, however, difficulty is experienced in obtaining sufficient material, as the cervix becomes small, hard, and covered with a necrotic layer of detritus.

Figures 4, 5, 6, and 7 are photomicrographs of sections obtained from specimens taken at intervals during the course of treatment from Case 6. Figure 4 shows untreated squamous-cell carcinoma, figure 5, beginning nuclear alterations in the cancer cells; figure 6, far advanced cellular degeneration and vacuolation; figure 7, the final stage in which cell detritus, fibrin, and amorphous material predominate.

The first cellular changes are noted about ten days after the initial treatment. Rapid disintegration of the surface of the growth does not become apparent before three weeks have elapsed.

From observations made upon readily accessible carcinomata (massive carcinomata of the vulva, two cases), it has become evident that 1 to 1.5 cm. is about the maximum depth of cancer tissue at which the rays of 120 mgm. of radium exert their effect. Beyond this distance, the cancer cells appeared to be unaltered, even after four or five prolonged exposures. This, of course, does not preclude the possibility that large quantities of radium, 500 to 1000 mgm., may perhaps exert a far deeper action. The majority of those who employ radium in pelvic disease, however, appear to agree that from 50 to 100 mgm. is the optimum amount to use.

The uterus removed from case 1, after eight radiations had been given, was cut in interrupted serial sections. In only two minute areas were spots suggestive of degenerating carcinoma cells found. Figure 8 shows the carcinoma before treatment, and figure 9 the suggestive areas; this section was at a level well above the fornix at a distance of at least 3 or 4 cm. from the tip of the cervix.

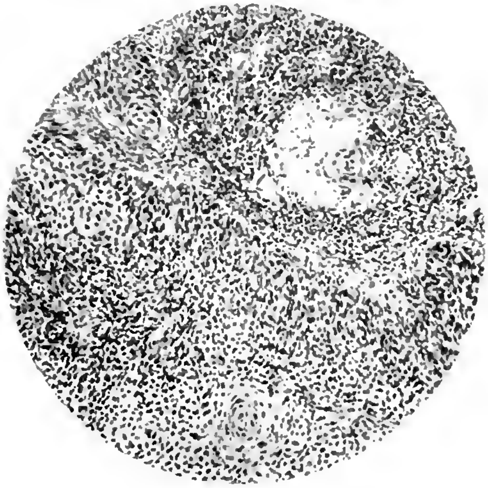

FIG. 4. M. M., JUNE 6, 1916
Squamous-cell carcinoma before treatment.

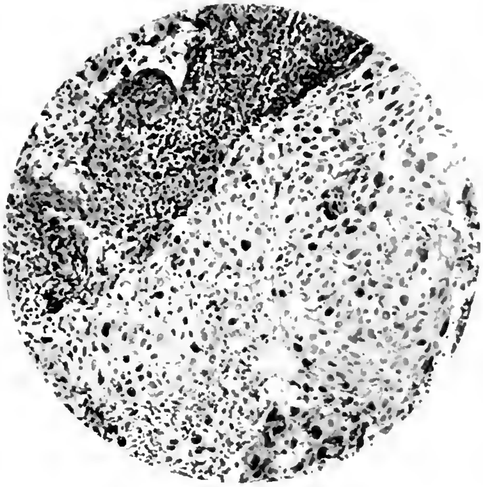

FIG. 5. M. M., JUNE 14, 1916
Same eight days after 1000 mgmhrs. of radiation. Note swelling of cells; enlargement and diffuse staining of nuclei.

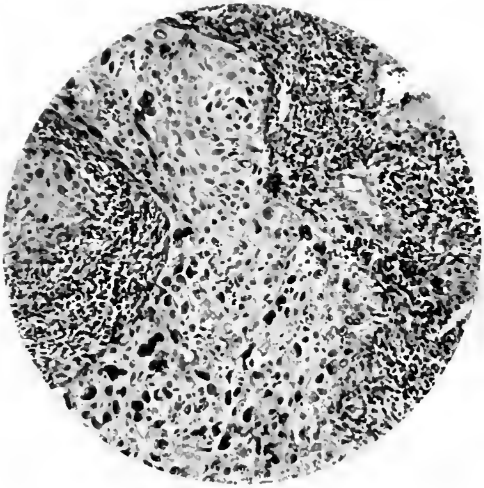

FIG. 6. M. M., JUNE 23, 1916

Same seventeen days after beginning of treatment, 900 additional mgmhrs. having been given. Note vacuolation of cells and increase in number of poorly staining nuclei.

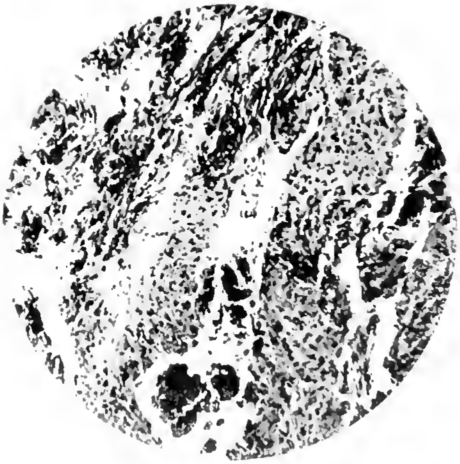

FIG. 7. M. M., JULY 3, 1916

Same twenty-seven days after beginning of treatment, 4200 mgmhrs. of radiation. Note purulent exudate containing an occasional degenerating cancer cell.

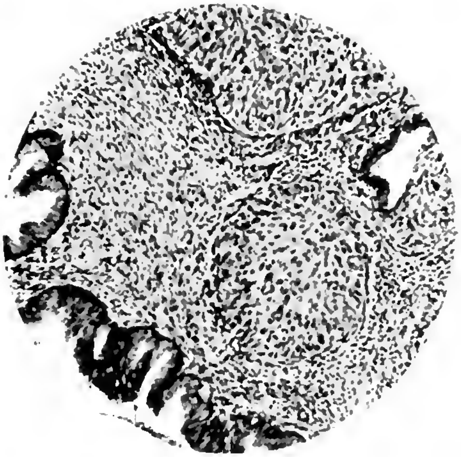

FIG. 8. P. G., FEBRUARY 11, 1916

Squamous-cell carcinoma in close proximity to cervical glands, before treatment.

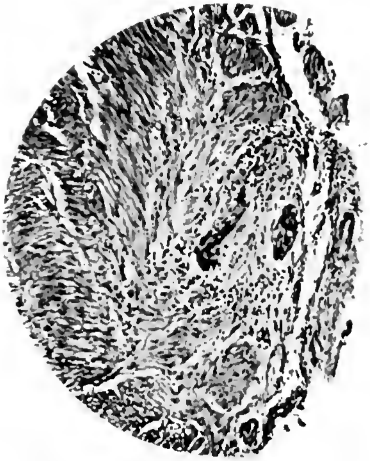

FIG. 9. P. G., APRIL 3, 1916

Section from uterus removed per abdomen, seven weeks after beginning of treatment (five radiations aggregating 4600 mgmhrs.). The entire uterus was cut and examined. The sole suspicious spots found are the two areas shown in the photomicrograph.

In view of the fact that the direct action upon cancer cells of the 120 mgm. of radium, as applied by the writer, does not manifest itself—when judged by histological criteria—beyond a distance of 1 to 1.5 cm., and yet at least temporary curative effects are observed over far wider areas *in the region of the cervix*, the writer is led to conclude that some other local factor must come into play. This local factor, in the case of cervical carcinoma, is probably supplied by the large quantity of connective tissue (parametria) which radiates from the cervix in all directions. Under the influence of the rays the connective tissue contracts, hardens, and perhaps proliferates. As a result, the lymphatics and smaller blood vessels are permanently blocked, and the dense scar produces a condition of "starvation" of the growth, a condition which has, at times, been obtained by surgical means (ligation of the internal iliac arteries). Further study is necessary and additional proof must be adduced before this suggestion can be accepted as more than a working hypothesis.

Histologically, carcinoma cells may appear normal and active, while biologically their growth and mitotic activity may either be inhibited or dead. This is especially true if the carcinomatous tissue is removed within eight to ten days after radiation. Figure 10 shows a sagittal section of the uterus removed in case 5 ten days after the first radiation had been given. The cells, when viewed with a high power (fig. 11) appear to be unchanged. Yet, though the vagina was cut across very close to the growth, no tendency to local recurrence was observed in the four weeks elapsing between operation and resumption of treatment.

Many further observations of interest and value can doubtless be made by close and persistent scrutiny of local excisions obtained during the course of treatment and of operative specimens obtained after preliminary radiation.

The following conclusions are warranted in the present state of our knowledge:

1. Radium is our best palliative measure in inoperable carcinoma of the cervix.
2. Far advanced cases may be treated with radium.

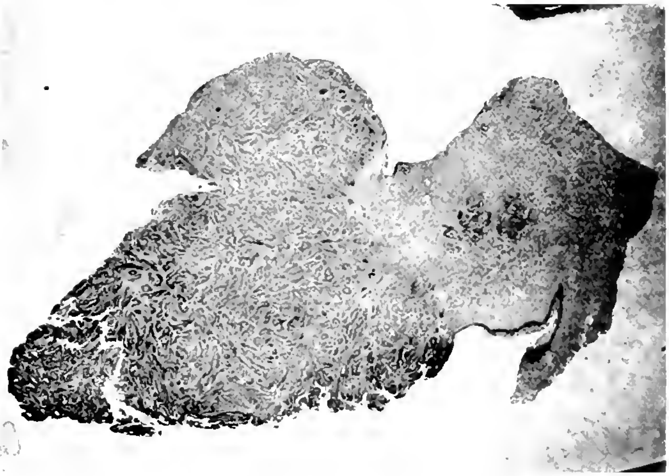

FIG. 10. F. W.

Sagittal section of the cervix of uterus removed nine days after the first radiation. Microscopically the cells appear unchanged and macroscopically the cervix looks infected and sloughy.

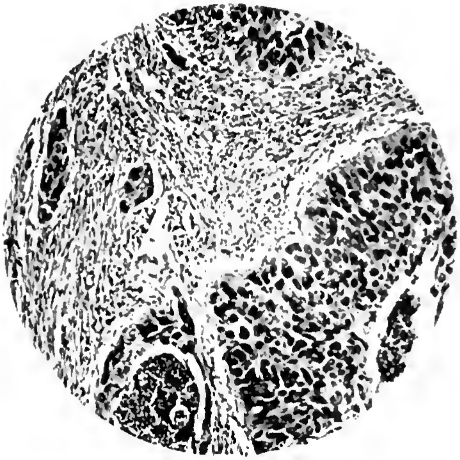

FIG. 11. F. W., MAY 1, 1916

Section from cervix of uterus removed per abdomen nine days after beginning of radiation (two radiations aggregating 2900 mgmhrs.). The cancer cells (1 cm. from the surface) as yet show no changes, though their power of multiplication may be inhibited or destroyed. Higher power of figure 10.

3. Radium not only rapidly relieves the pain, hemorrhage, and discharge, but indirectly also improves the general health and condition.

4. The minimum quantity of radium substance needed is 50 mgm.

5. "Border line" cases or operable cases should be submitted to operation after a short preliminary course of radiation. Good primary results may then be expected from simple total hysterectomy.

6. Operated cases should be subjected to postoperative, prophylactic radiation, beginning not later than four weeks after operation.

7. The technique of radium treatment of cervical cancer is simple and easy to learn.

In conclusion, a word of warning must be given against the building of undue hopes upon this recent addition to our weapons in the fight against cancer. The above report of early results obtained agrees in the main with the favorable results reported by many others, and shows that radium is a wonderful *palliative*. Whether the *final results* will prove that radium can give a permanent cure of cancer is a mooted question. Judging from the limited penetrating power of the rays and the variation of resistance of different cancers, it seems probable that numerous disappointments will occur, and that in many cases positive harm will be done by enthusiasts who refuse to submit operable cancers to surgical operation.

HISTORIES

Case 1. A. E., sixty years old. Eight treatments beginning December 3, 1915, ending June 27, 1916, total 9242 mgmhrs. Ankylosed right hip, severe cardiac. Three children. Menopause ten years ago For two years foul discharge, four weeks bleeding. Cervix composed of a friable, hard lobulated mass involving fornices and right parametrium. Microscopically squamous-cell carcinoma.

After being well for eight months, with exception of rectal tenesmus, is now losing weight and strength. No local recurrence or metastases found.

Case 2. D. Z., forty-eight years old. Repeated cauterizations, tying off of vaginal branches of uterine arteries. Last cauterization December 28, 1915. Crater from cauterizations, left parametrium infiltrated. Microscopically carcinoma. Five radiations, beginning January 4, 1916, ending March 31, total 5029 mgmhrs. At present feels and looks well. No general or local signs of recurrence.

Case 3. D. E., thirty-three years old. Radical operation for carcinoma of cervix ten months before admission; reoperated for vaginal recurrence July, 1915. Since then repeated vaginal hemorrhages. Again operated December, 1915. On admission February 3, 1916, vault occupied by infiltration extending into both parametria and on right side extending almost to mucosa of rectum. No material for microscopic examination. Six radiations beginning February 3, 1916, ending July 18, total 6286 mgmhrs. At present feels and looks well, no infiltration or signs of recurrence or metastases.

Case 4. P. G., forty-two years old. Two abortions, operated at Mt. Sinai Hospital in 1912 for intraligamentous cyst, vaginal bleeding daily for three months. A red granular mass has substituted the posterior lip of the cervix infiltrating the right and posterior vaginal fornix (possibly still operable). Microscopically squamous-cell carcinoma. Five radiations beginning February 11, 1916, ending March 21, total 4629 mgmhrs. Total abdominal hysterectomy April 3, without excision of parametria. Uterus embedded in glistening white stony hard scar tissue. Uneventful convalescence except ureterovaginal fistula for four weeks. At present looks and feels well. No signs of local or general recurrence.

Uterus showed only two small microscopic areas suggestive of cancer cells.

Case 5. F. W., fifty years old; menopause three years ago, bleeds daily for eight months, has lost weight and strength. Entire cervix, especially posterior lip, is hypertrophic, uneven, bleeding freely to touch, parametria free (operable?). Microscopically squamous-cell carcinoma. Two radiations beginning April 22, 1916, ending April 28, total 2984 mgmhrs. May 1, complete hysterectomy without excision of parametria. Microscopically the carcinoma cells are unchanged (figs. 10 and 11). Three additional radiations given, beginning May 29, ending July 29, total of 8132 mgmhrs. in five sessions. At present feels and looks well. No signs of local or general recurrence.

Case 6. M. M., sixty-two years old. Eleven children, menopause fifteen years ago, foul discharge for two years, bleeding ten weeks.

Obese, emphysematous, potatrix. The cervix consists of a bulging mass, extending into left fornix and infiltrating the upper part of the recto and vesicovaginal septa. Microscopically squamous-cell carcinoma. Six radiations, total 8077 mgmhrs., beginning June 6, 1916, ending July 27. Has had severe attack of articular rheumatism. No local signs of recurrence. At present is losing weight and strength, liver enlarged and nodular.

Case 7. W. M., fifty-two years old. No pregnancies, menstruation regular until one year ago, since then meno- and metrorrhagia; lost twenty pounds in six months. Cauterized repeatedly for inoperable squamous-cell carcinoma before admission, but without relief. Crater infiltration of all vaginal walls to within 3 cm. of introitus, especially in vault, marked in vesicovaginal septum. Four radiations beginning August 16, 1916, ending October 5, total 10,905 mgmhrs. At present looks and feels well. Upper vaginal vault hard, uterus retroflexed, not freely movable. By rectum vaginal vault feels free, the right parametrium is doubtful.

Case 8. L. Z., fifty-seven years old. Ten pregnancies, menopause thirteen years ago, has been spotting eight months. Upper third of vagina infiltrated, vault rigid, cervix crater like, infiltration of vesico- and rectovaginal septum. *X*-ray shows pathological fracture of neck of right shoulder. Microscopically squamous-cell carcinoma. Four radiations beginning September 11, 1916, and to be continued, total to date 6070 mgmhrs. Crater (November 11) smaller, no bleeding, much improved in health. *X*-ray shows progress of arm lesion.

Case 9. C. Prophylactic radiation after Wertheim hysterectomy (May, 1915) 1660 mgmhrs. At present well.

Case 10. C. G. Prophylactic radiation after Wertheim hysterectomy June, 1915, 1880 mgmhrs.

SPONTANEOUS EPITHELIOMA OF THE FOWL

ADAH I. BAIRD

From Columbia University, New York, George Crocker Special Research Fund, F. C. Wood, Director

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Spontaneous carcinomata and keratinizing epitheliomata of birds, especially of the common fowl, are not particularly rare. Thus, Wernicke (1) has reported several cases and given references to many more; while among more recent authors, Bürger (2) has described three adenocarcinomata of the ovary in hens, Fujinami (3) thirty-two carcinomata in the same species, affecting chiefly the ovary, Schöppler (4) a carcinoma of the stomach, and so on.

The tumor to be described was found, at autopsy, in a common fowl of the Plymouth Rock strain. On examination of the abdominal cavity, the right kidney was seen to be protruding from the bed in which it normally lies. It was shelled out from below upwards and a tumor was found adherent to the posterior surface. Owing to the soft structure of the avian kidney, it was difficult to determine whether the mass was a direct outgrowth of the kidney or not.

In the gross, the tumor was pale, the tissue was firm; it formed an elongated, encapsulated mass, measuring 40 x 30 mm. Portions of the tumor were fixed in Zenker's fluid, sectioned, stained with Delafield's hematoxylin, and counterstained with eosin.

On microscopical examination, the tumor proved to be a squamous-cell epithelioma. The parenchyma of the growth was composed of interlacing strands of epithelium, in some places developing into cylindrical alveoli lined with a single layer of cells. In other areas, the strands were solid and could scarcely be distinguished from the richly cellular stroma. In still other portions of the tumor, large and small cysts had developed from

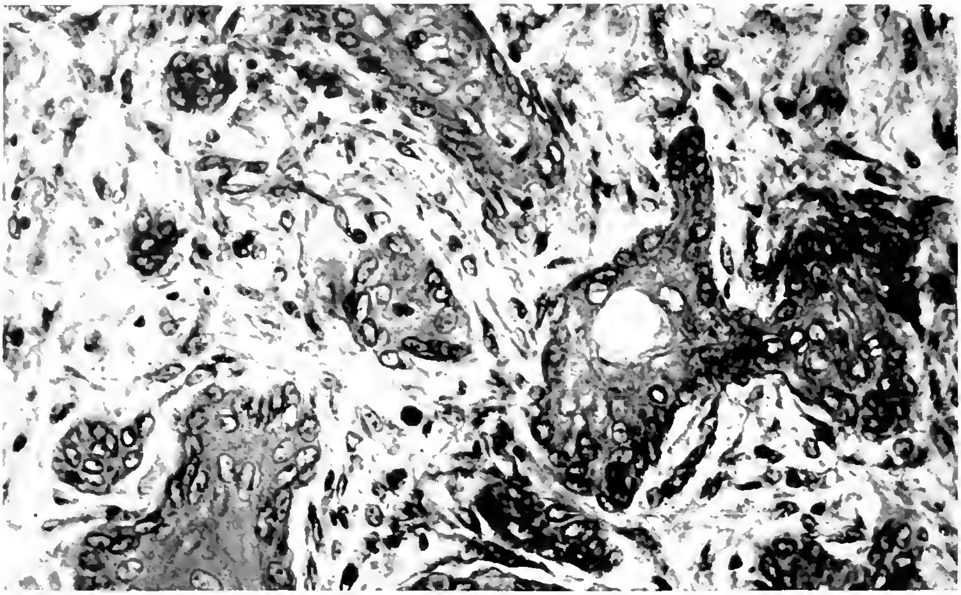

FIG. 1. SQUAMOUS-CELL PORTION OF TUMOR. × 400

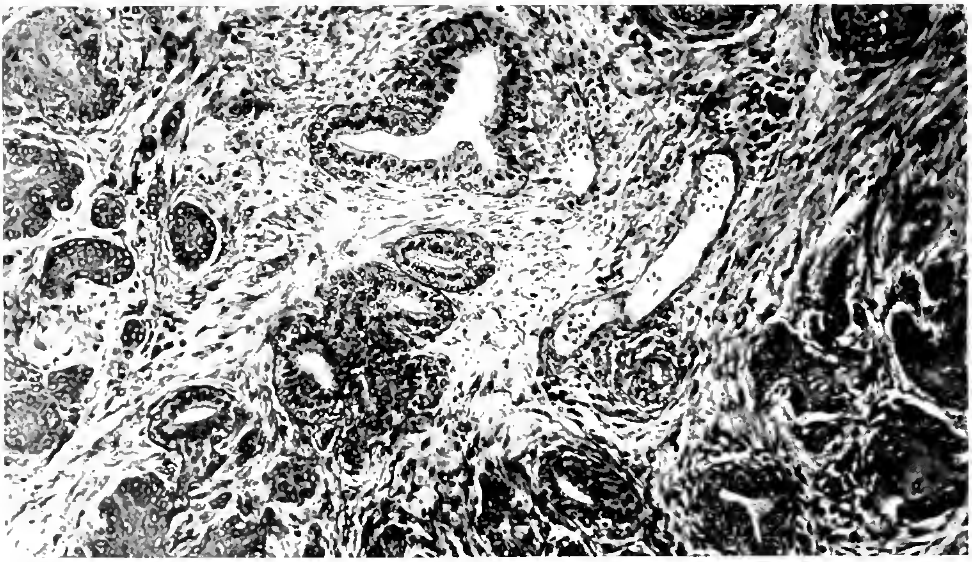

FIG. 2. GLANDULAR TUBULES IN TUMOR. × 400

the keratinization of large masses of epithelium with death of the cells and the formation of cavities; into some of these cavities the connective tissue stroma had secondarily penetrated. Where contact between dead epithelium and connective tissue occurred, there was a large number of phagocytic giant cells. The stroma was highly vascular and very cellular, and contained large numbers of small spindle cells. It was infiltrated with many leucocytes and plasma cells, the leucocytes being, in the main, of the mononuclear variety. Blood vessels were fairly abundant and very well marked, and were filled with nucleated red blood corpuscles. Mitotic figures were infrequent, either in the stroma or in the tumor proper. The epithelial cells, when in alveolar form, showed masses of mucoid secretion or of a substance staining pink with eosin and resembling the material frequently found in resting glands of epithelial organs, such as the breast. There was but little variation in the general

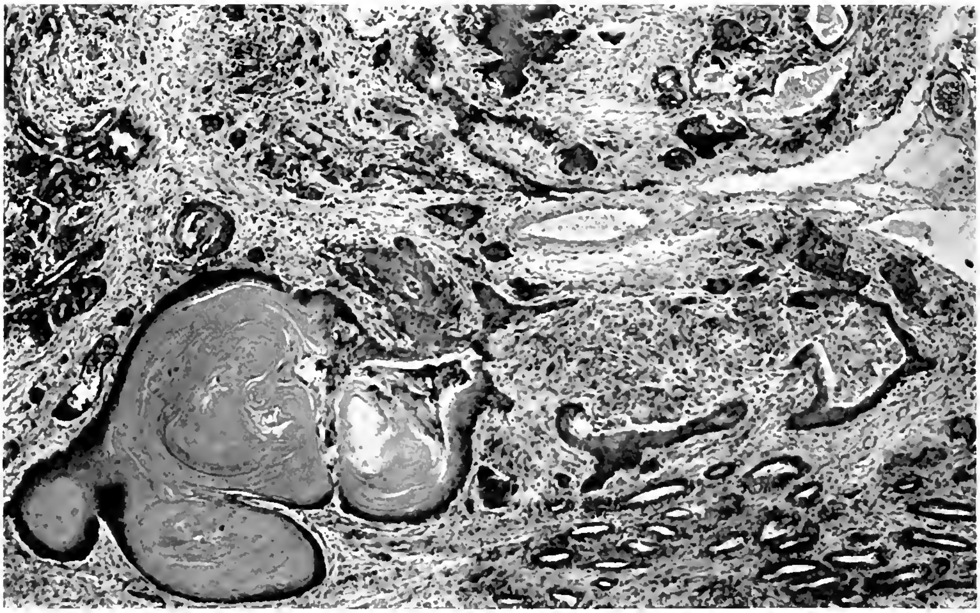

Fig. 3. Areas of Keratinization in Tumor. × 75

topography of the tumor; some portions contained a little more of the tubular elements, others a little more of the solid. A relatively small part was made up of cysts or of areas of necrosed epithelium.

The tumor did not contain any microscopic structures wholly characteristic of the kidney or its pelvis, so that its origin must probably be considered to have been from a displaced embryonic epithelial remnant of an early stage renal anlage.

The growth was transplanted into one hundred chickens of the same strain. In fifty, 0.03 gram of tumor was planted into each pectoral muscle with a Bashford needle. In the remaining fifty, in addition to the inoculation in the pectoral muscles, the same amount of tumor was placed in the peritoneal cavity. All these chickens were allowed to live, and finally came to autopsy after from four to twenty weeks. Complete autopsies were made, and in not a single bird was a growth found.

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(1) WERNICKE: Ztschr. f. Krebsforsch., 1911, x, 168.
(2) BÜRGER: Ztschr. f. Krebsforsch., 1914, xiv, 526.
(3) FUJINAMI: Gann., 1908, ii.
(4) SCHÖPPLER: Ztschr. f. Krebsforsch., 1913, xiii, 332.

OBSERVATIONS UPON THE EFFECTS OF RADIUM ON TISSUE GROWTH IN VITRO

FREDERICK PRIME

From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director

During the last few years much has been written both for and against the usefulness of radium as a therapeutic agent in the treatment of malignant growths. The observations along this line have been chiefly clinical, made, that is, upon tumors in man, where it was impossible to control the results with any degree of accuracy. Each investigator has developed a different method of applying and filtering rays, so that much confusion has arisen in regard to the most satisfactory method of applying these rays and the manner of employing them in order to obtain the best results.

Two years ago investigations were begun along this line in the Crocker Fund laboratory, using transplantable animal tumors, in which the results can be controlled with a far greater degree of accuracy than is possible in the human subject. The outcome of these experiments has been published in full elsewhere (1).

Little attention has been paid in the work already done, however, to the effect of radium upon the isolated cells of mammalian tissue. Yet it is here that the action of the radium rays is exerted for good or ill, and it is essential to know just what takes place within the confines of the cell exposed to the influence of this element. It was in order to clear up this phase of the question, therefore, that the following work was undertaken.

The employment of *in vitro* cultures of cells growing in plasma offers a convenient method for observing growing tissue, and this, therefore, was chosen as the most suitable means for studying the changes produced by radium upon the individual cells.

In all the work which has appeared since radium has been obtained in sufficient quantities to allow of its general use, practically no observations have been made previous to 1914 upon the action of radium rays on living mammalian tissue grown outside the host. As early as 1911, however, Wedd and Russ (2) reported a series of experiments in which a transplantable tumor was removed from the mouse in which it had grown, kept moist between mica sheets during exposure to radium, and then inoculated into mice; it was found that no growth resulted from the grafts, provided they had been radiumized for a sufficient length of time. Several years later, Wassermann (3) published the results of his work along the same line. He, however, exposed pieces of tumor tissue suspended in Ringer's solution to the radium rays and inoculated these fragments into animals, but did not try to grow the cells outside of the body. He formulated from his results the hypothesis, that following radium therapy there results a nuclear but not a cellular death. No observations were made, however, on the growing cells themselves, a circumstance which rendered his deductions of less value than they might otherwise have been. In this same year, however, Price Jones and Mottram (4) undertook to expose pieces of transplantable mouse and rat carcinomata to the action of radium rays. After the exposure, plasma cultures were made of the tissue and twenty-four hours later there was observed an extrusion of spindle cells which had been uninfluenced in their ameboid activity by the beta or gamma rays. The alteration in the power of these cells to divide, as shown by the decrease in the number of mitotic figures, was regarded by them, however, as evidence of a profound effect exercised by the radium rays upon the growth of tumors. Their results partially corroborated Wassermann's hypothesis concerning the increase in the size of the growth, which he had suggested was due to an ameboid outwandering of the cells and not to any true mitotic division. In other words, Wassermann believed, though he had not made any observations upon tissue growing *in vitro*, that the proliferative power of the cells was destroyed by the action of the radium rays, but that the cells were not killed, being left, rather, in a purely vegetative condition. Whether this was the case or not still remained undecided, and the evident importance of the question stimulated the undertaking of further experiments along the same line, which are here reported.

The first tissue used was that of the embryo chick heart, as it can be kept alive and growing for a long period by the method of culture set forth by Carrel (5). The fact that this muscle continues to beat for a long time after its removal made it peculiarly favorable for this work; for were the heart to continue to beat this would be a conclusive indication that the culture was still alive, even though there was no evidence of an outgrowth of those new cells which are regarded by Carrel and most investigators as connective tissue elements. The method used was simple, and followed fairly closely the technic already described by Carrel and others. Hearts were removed from chicks which had been incubated for from five to seven days, cut into small pieces about 0.001 gram in weight, and some two or three dozen such fragments suspended in a hanging drop of Ringer's solution on a cover slip sealed with paraffin over a hollow slide. The cultures to be treated with radium were then covered with 0.4 mm. of brass, upon which the radium tubes were placed, and thus exposed to the action of 100 mgm. of radium for one-half and two hours respectively. In a previous series of experiments (1), it had been found that the lethal point of small pieces of tissue, where the unfiltered rays were concerned, was twenty, fifteen, and ten minutes for 17, 83, and 100 mgm. of radium respectively. When the alpha and soft beta rays were removed by filtration with 0.4 mm. of brass, three hours, one hour, and forty-five minutes respectively, were required for 17, 83, and 100 mgm. to kill; whereas when only the gamma and secondary beta rays were employed, twenty hours were necessary for 17 mgm. of radium, and about seven hours for both the 83 and 100 mgm., to cause death.

The two exposures to 100 mgm. of radium mentioned above for one-half and two hours when both beta and gamma rays were used was therefore a sublethal and a lethal dose respectively. After exposure, each piece of tissue was removed from the

Ringer's solution, and cultures of them were made in slightly diluted chicken plasma, controls being accorded the same treatment in all cases. The cultures were all kept in an incubator at 37°C., and daily observations made as to their condition. At the end of the first twenty-four hours, there was found to be a profuse outgrowth of connective tissue cells in all the *in vitro* cultures, both treated and controls, while the muscle in all cases was actively beating. At the end of forty-eight hours, while the pulsations were not so active, the connective tissue cells showed a further increase in area. At the end of forty-eight to seventy-two hours, the tissue cultures were removed from the plasma and washed in warm Ringer's solution, after which they were put into fresh plasma, and examined at the end of a second period of twenty-four hours. In all the cultures there was again an increase in the superficial area of the tissue, without any noticeable difference in the outgrowth of cells in treated and controls. The number of heart fragments which were pulsating, however, was usually less than in the first generation of plasma cultures. After forty-eight hours more, these were again transplanted, and in this third generation at the end of twenty-four hours a marked difference was observed. The controls, as well as the cultures which had been exposed to a sublethal dose of radium, still showed a good connective tissue outgrowth; but where the cultures had been exposed to the action of the radium for two hours there was a marked reduction in the area of this growth. Usually one or two cultures showed a slight outgrowth, though in the majority none was apparent; but in many of those which showed no connective tissue proliferation, the heart muscle was still beating, proving that they were at least alive. In the fourth generation, no outgrowth at all was found where there had been a long exposure to radium, but some heart fragments were still pulsating; in this generation, too, there was seen a change in those cultures which had had a sublethal exposure. In those specimens which exhibited outgrowth the cells were not so numerous, nor was the increase in area nearly so great as in the controls, and many cultures which were beating showed no evidence of outgrowth.

In the fifth generation, there was found to be practically no outgrowth in any of the treated cultures, though many of the radiumized muscle cells were still beating, whereas the controls were still surrounded by vigorously growing connective tissue cells, and many more pieces of heart tissue were beating than in the treated cultures. From this time up to twenty-one days, when the cultures were discarded, no change was apparent. The controls continued to be surrounded by connective tissue cells and many of the bits of heart muscle were pulsating; no late outgrowth was ever found in the radiumized tissue, however, though several fragments of heart muscle were observed to beat occasionally. This experiment shows that growth of the connective tissue was stopped by the radium though the heart muscle continued to functionate.

Following these experiments upon the chick embryo heart, tumor tissue was employed; here more accurate observations could be made, as photomicrographs of the growing cells could be readily obtained.

The tumor was cut into small pieces about 0.001 gram in weight, which were either planted in plasma and exposed in an incubator at 37°C. to the action of the radium rays, or suspended in cold Ringer's solution while being radiumized, and then plasma cultures were made of them, an equal number of control plants from unexposed tumor being made in each series. This latter method was the one most constantly employed, as it was deemed a somewhat safer and more accurate procedure, for the reason that the rays might conceivably cause some change in the plasma medium, which, in its turn, might have some action upon the cells themselves. Another factor in favor of this method was that the tissue did not begin to grow while being exposed to the radium, as was found to be the case when they were planted directly into plasma and the exposure to the radium made while they were in the incubator.

The tumors used were a mouse carcinoma, Crocker Series No. 11, and a mouse sarcoma, Crocker Series No. 180. Both of these tumors had been under observation for many months in the laboratory and their action and method of growth well determined. The rays employed were derived from 47, 83, 100, and 130 mgm. of radium element. These were used unfiltered so that only the alpha rays were eliminated, slightly filtered so that the alpha and soft beta rays were removed, or with such a degree of filtration that nothing but the gamma and secondarily produced beta rays reached the tissue. The length of time during which the tissue was exposed to the action of the radium varied, according to the amount of filtration used, from a quarter of an hour to fourteen hours.

The tissue, which had been kept in the ice-box during the exposure to the radium, was immediately thereafter planted into plasma and put into an incubator at 37°C. In this manner, accurate observations could be made and photomicrographs taken when necessary, every precaution being employed to prevent the cultures from becoming unduly chilled while being examined. The greatest technical difficulty encountered was in obtaining a sufficient amount of mouse blood to supply the necessary plasma, and to keep it from coagulating before all the cultures in each series of experiments had been made. It was found that 1 and 2 cc. Luer syringes, sterilized in the autoclave, kept cold in a refrigerator, and coated just before use with ice-cold sterile olive oil aspirated after attachment of the needle, did not induce clotting of the blood. The mice were prepared by removal of the hair from the thoracic region, after which they were etherized, and the skin rendered sterile by iodine. The needle was plunged directly into the heart and from 0.5 to 1 cc. of blood aspirated. This was put into ice-cold paraffined test tubes, and centrifugalized, when the supernatant plasma was drawn off and put into cold tubes. It was then diluted 1 to 3 with cold Ringer's solution and the cultures made in this medium. By this method there was usually a working period of from one to one and one-half hours in which to make the cultures before the plasma coagulated.

The exposure to unfiltered radium rays was made only a few times, as it is not practicable as a rule to employ radium in this manner in treating human beings on account of the burning which follows its use. In cultures of $\frac{180}{19\,D}$, both of the specimens

NUMBER	TUMOR	AMOUNT RADIUM	CHARACTER OF RAYS	LENGTH OF EXPOSURE	RESULT	OBSERVATIONS ON STAINED SECTIONS
		mgm			*per cent growing*	
180 19D A	Sarcoma	47	All beta and gamma	Lethal, 2 hours	42	Stained sections show no mitotic figures, and photomicrograph shows limitation of outwandering cells.
B	Sarcoma	47	Hard beta and gamma	Lethal, 2 hours	100	No mitotic figures but cells look more normal than in A. Profuse outwandering cells.
D	Sarcoma	83	Gamma	Sub-lethal, 6 hours	95	No mitotic figures. Growth similar to B.
C	Sarcoma	Controls	for A, B, D		99	Mitotic figures in outwandering cells. Growth profuse.
11 35A A	Carcinoma	100	Hard beta and gamma	Sub-lethal, ½ hour	57	Occasional mitotic figure in outwandering cells.
B	Carcinoma	100	Hard beta and gamma	Lethal, 2 hours	66	No normal mitotic figures. Cells outwandering similar to A.
C	Carcinoma	Controls	for A, B		77	Many mitotic figures present.
11 32 A_2	Carcinoma	130	Gamma	Sub-lethal, 2 hours	100	No mitotic figures Many outwandering cells
A_3	Carcinoma	130	Gamma	Sub-lethal, 3 hours	100	No mitotic figures. Fair outwandering of cells.
B_5	Carcinoma	130	Gamma	Sub-lethal, 5 hours	100	No mitotic figures. Slight outwandering of cells.
C_2	Carcinoma	130	Gamma	Lethal, 8 hours	100	No mitotic figures. No outwandering cells.
C_1	Carcinoma	Control	for A_2, A_3 B_5, C_2		100	Many mitotic figures. Many outwandering cells.
180 18G 3	Sarcoma	130	Gamma	Sub-lethal, 5 hours	100	Profuse outwandering of cells. Transplanted for three generations without showing mitotic figures.
1	Sarcoma	Control	for 3		100	Profuse outwandering of cells. Transplanted for three generations and shows mitotic figures

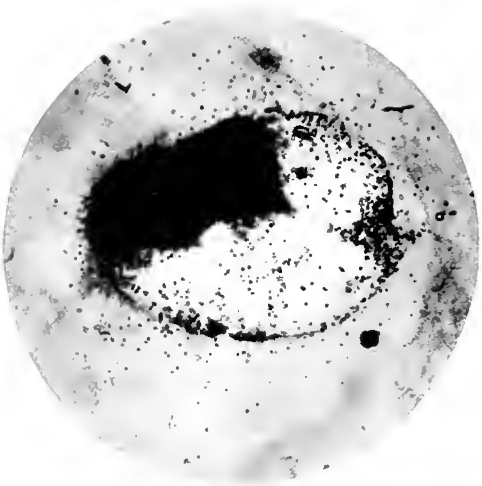

FIG. 1. TISSUE CULTURE $\frac{180}{19\ D}$ A

No filtration. 47 mgm. of radium for two hours. × 25.

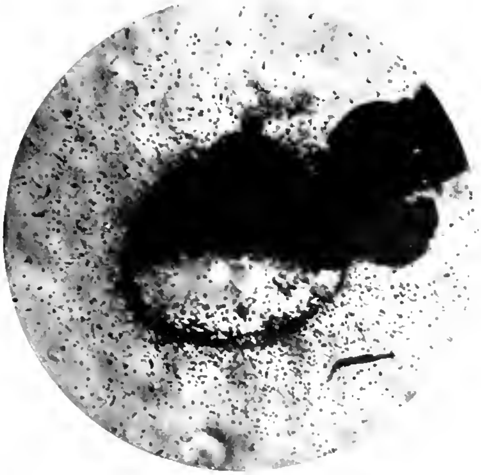

FIG. 2. TISSUE CULTURE $\frac{180}{19\ D}$ B

Hard beta and gamma rays from 47 mgm. of radium for two hours. × 25.

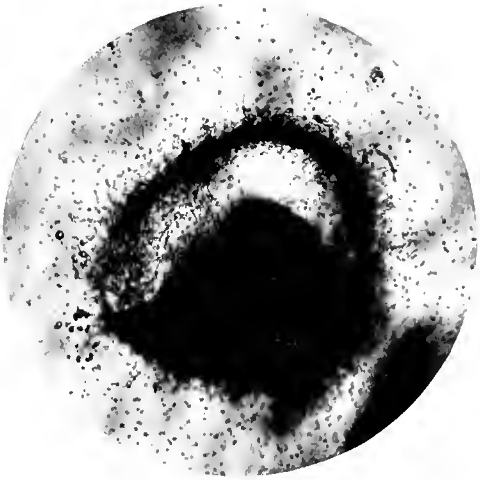

Fig. 3. Tissue Culture $\frac{180}{19\,D}$ D

Gamma rays from 83 mgm. of radium for six hours. × 25.

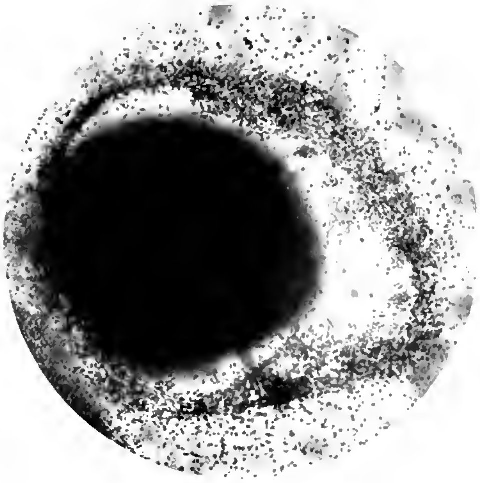

Fig. 4. Tissue Culture $\frac{180}{19\,D}$ C

Control for A-B-D (Figs. 1, 2, 3). × 25.

treated with filtered rays show almost as marked cell proliferation as the control cultures, whereas that treated with the unfiltered rays shows only slight proliferation in the cells (figs. 1, 2, 3, 4); but none of the treated cultures shows any mitotic figures on standing. In $\frac{11}{36A}$ where a sublethal as well as a lethal dose of radium was given, both of the treated specimens showed more proliferation than did the control (figs. 5, 6, 7); but the culture given the larger dose of radium showed no mitoses when stained, whereas the control and the culture given a sublethal dose did (figs. 8, 9, 10).

FIG. 5. TISSUE CULTURE $\frac{11}{36A}$ A

Hard beta and gamma rays from 100 mgm. of radium for one-half hour. × 25.

In cultures of $\frac{11}{32}$, after a series of exposures (varying from one to eight hours) to the rays from 130 mgm. of radium filtered through 1.2 mm. brass and 5 mm. filter paper, which eliminated all but the gamma and secondarily produced beta rays (6), it was found that at the end of twenty-four hours there was a marked outgrowth of cells in the treated specimens as well as

FIG. 6. TISSUE CULTURE $\frac{11}{36A}$ B

Hard beta and gamma rays from 100 mgm. of radium for two hours. × 25.

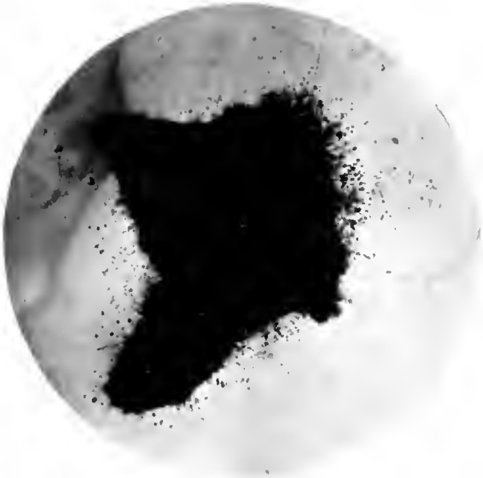

FIG. 7. TISSUE CULTURE $\frac{11}{36A}$ C

Control for A-B (Figs. 5, 6). × 25.

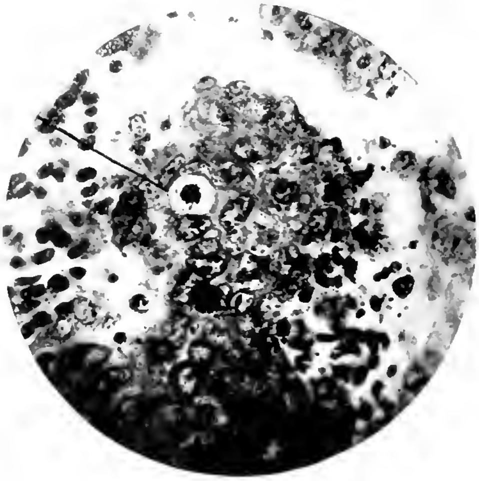

FIG. 8. STAINED SECTION OF TISSUE CULTURE $\frac{11}{36\,A}$ A

Sublethal dose of radium. One mitotic figure. × 1000.

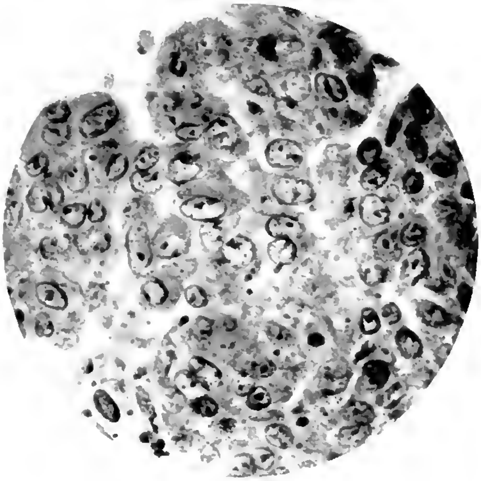

FIG 9. STAINED SECTION OF TISSUE CULTURE $\frac{11}{36\,A}$ B

Lethal dose of radium. No mitotic figures. × 1000.

in the controls. Those treated for two and three hours with radium show at least as large an outwandering as is found in the control; while where the treatment had been prolonged to five and eight hours there is not so marked a tendency for the cells to wander out as is shown in the photomicrographs (figs. 11, 12, 13, 14, 15). Stained sections from the cultures and the control slides show mitotic figures in the latter but none in those treated with radium. This was corroborated by removing the tissue from the slides and inoculating it into mice; the controls grew and produced good sized tumors in the majority of cases, while there was no growth from those plants which had been subjected to either a lethal or sublethal exposure to radium rays, and which, even though they had shown an outwandering of cells in the plasma cultures, had shown no mitotic figures in stained sections. In those cases where the plasma cultures had been allowed to grow from twenty-four to forty-eight hours, and had then been removed from the plasma and washed in Ringer's solution, an outwandering of cells might often be observed in the radiumized specimens on culturing in fresh plasma into the second and

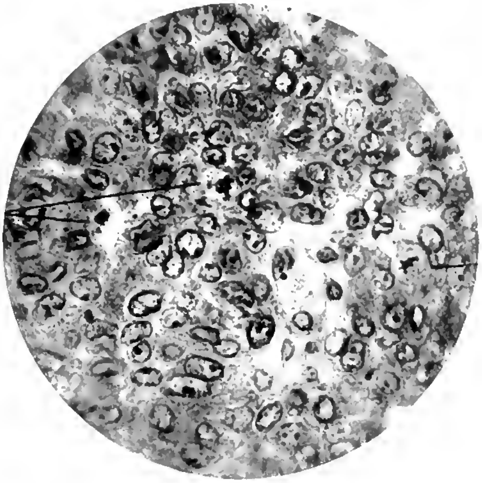

Fig. 10. Stained Section of Tissue Culture $\frac{11}{36\text{A}}$ C

Control. Numerous mitotic figures. × 1000.

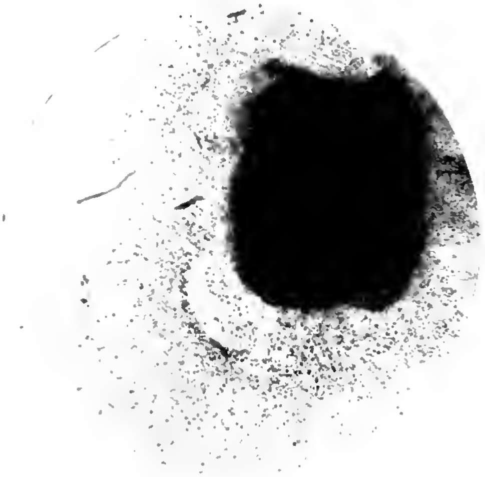

Fig. 11. Tissue Culture $\frac{11}{32}$ A_2

Gamma ray from 130 mgm. of radium for two hours. × 25.

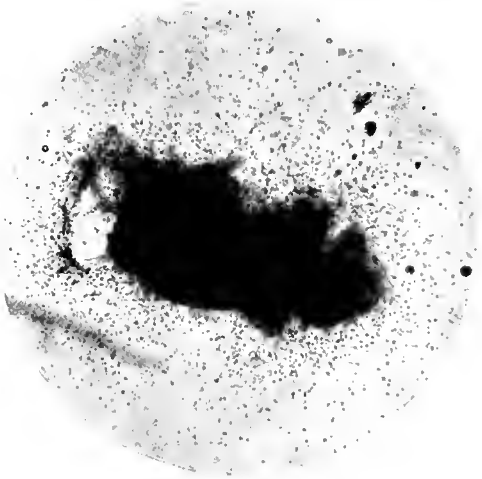

Fig. 12. Tissue Culture $\frac{11}{32}$ A_3

Gamma ray from 130 mgm. of radium for three hours. × 25.

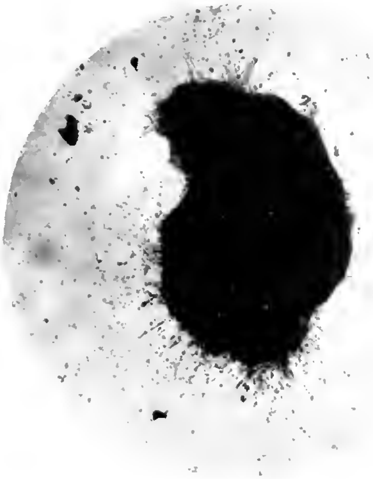

FIG. 13. TISSUE CULTURE $\frac{11}{32}$ B_5

Gamma ray from 130 mgm. of radium for five hours. × 25.

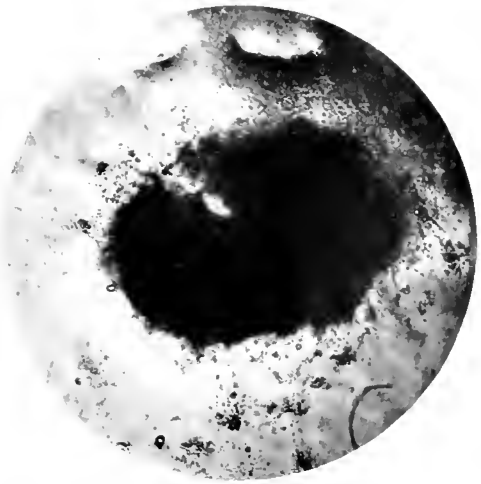

FIG. 14. TISSUE CULTURE $\frac{11}{32}$ C_2

Gamma ray from 130 mgm. of radium for eight hours. × 25.

third generations; but after that there was seldom any visible activity, though the control cultures showed outwandering cells with mitotic figures for a somewhat longer period of time. This may be seen in the accompanying photomicrographs of the growing plasma cultures. At the end of twenty-four hours (figs. 16, 17) the treated tissue which was given a slightly sublethal dose of radium shows an outwandering of cells quite as marked as is seen in the controls. When these tissues were transplanted into a second generation (figs. 18, 19) there was still a marked outwandering of cells, which in this series happened to be greater in the treated than in the control cultures, though this was not always the case. The third generation of transplanted cultures showed, however, practically no outwandering of cells in the treated cultures, whereas they were still marked in the controls (figs. 20, 21). Upon staining and sectioning these different generations of plasma growths, all the control sections showed the presence of mitotic figures in the outwandering cells, whereas none were found after careful study in the treated tissues.

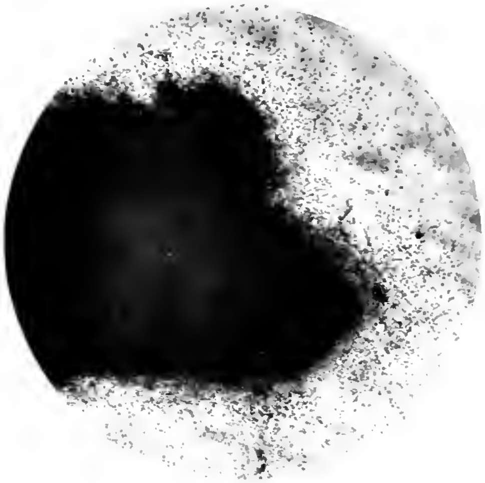

Fig. 15. Tissue Culture $\frac{11}{32}$ C_1

Control for A_2, A_3, B_5, C_2, (Figs. 11, 12, 13, 14). × 25.

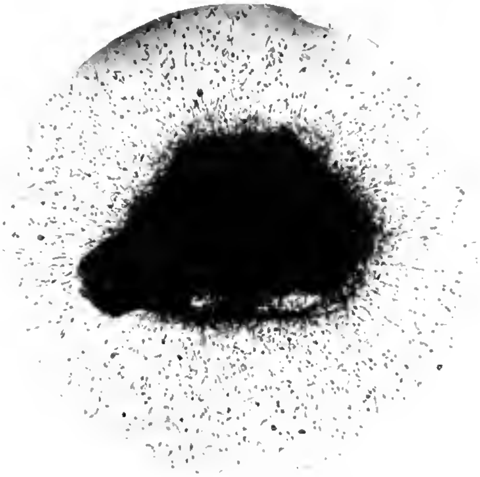

FIG. 16. TISSUE CULTURE $\frac{180}{18G}$ 3

Gamma ray from 130 mgm. of radium for five hours. × 25.

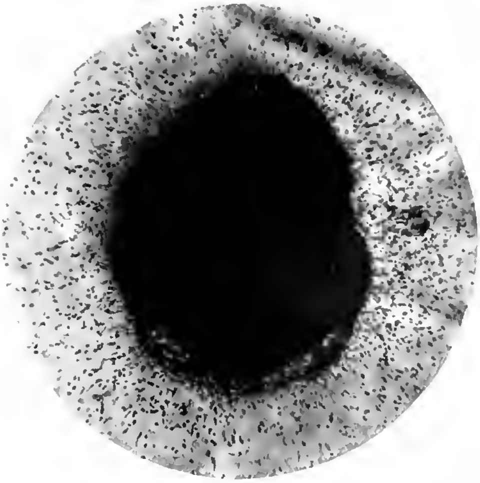

FIG. 17. TISSUE CULTURE $\frac{180}{18G}$ 1

Control for $\frac{180}{18G}$ 3 (Fig. 16). × 25.

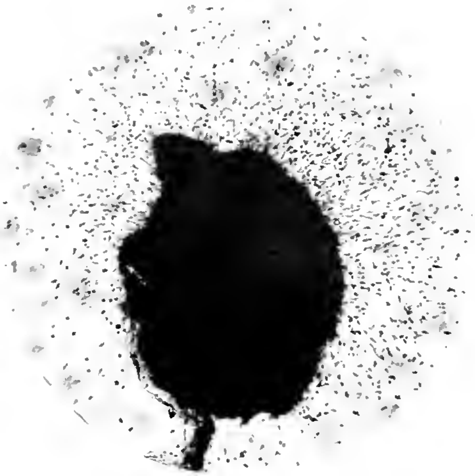

Fig. 18. Tissue Culture $\frac{180}{18G} A_2$

Second generation after treatment with gamma ray from 130 mgm. of radium for five hours. × 25.

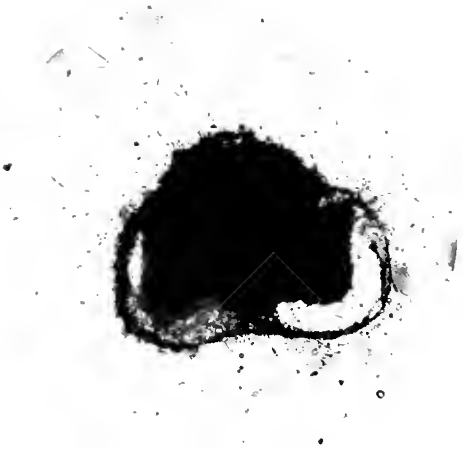

Fig. 19. Tissue Culture $\frac{180}{18G} A_1$

Second generation control for $\frac{180}{18\,G} A_2$ (Fig. 18). × 25.

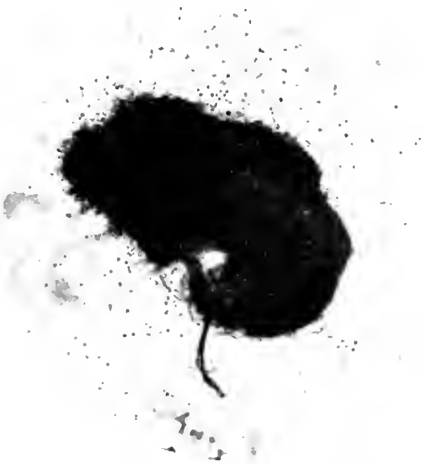

FIG. 20. TISSUE CULTURE $\frac{180}{18G}$ R$_3$

Third generation after treatment with gamma ray from 130 mgm. of radium for five hours. × 25.

FIG. 21. TISSUE CULTURE $\frac{180}{18G}$ C$_3$

Third generation control for $\frac{180}{18G}$ R$_3$ (Fig. 20). × 25.

The results thus obtained support in most respects our previous experiments (1) in which the treated tissues were inoculated into mice; but since tissue treated with sublethal doses of radium will grow when put into mice, it was surmised that tissue grown in plasma cultures, after being given sublethal doses of radium, might contain some mitotic figures among the outwandering cells, even if those treated with lethal doses did not. In fact, upon examining stained sections, a very few normal mitoses were occasionally found in the outwandering cells after sublethal doses, while in the control plasma cultures, abundant mitotic figures were found in the cells which had spread out from the main tissue. When a lethal dose of radium had been given, however, no mitotic figures were found in the outwandering cells, though an occasional cell was noted in which the nuclei showed a very atypical arrangement of the chromatin in what was apparently an abortive mitotic figure.

In order to conclude this work, a series consisting of treated and untreated plasma cultures was made and, after growth had developed, each culture was divided into two parts. The cells which had wandered out from the original tumor plant were carefully removed by a sharp cataract knife and inoculated into one set of mice, while the original piece of tissue from which the outwandering cells had been removed was inoculated into another group of mice. In a large series of experiments it was found when a lethal dose of radium had been given, even though there was a very pronounced outwandering of cells in the plasma, that when these cells were inoculated into mice as well as the original tissue neither produced tumors in a single instance. When a sublethal dose of radium was given, the original treated tissue when removed from the plasma grew in most instances, but the cellular outgrowths never produced a tumor in mice. When the same was done with the control cultures, however, both the original plants and the cellular outgrowth developed tumors in mice.

This raises the interesting problem, why the original tissue after exposure to a sublethal dose of radium can grow when introduced into mice, while its cellular outgrowth can not. The observation may possibly be explained by the very few mitotic cells found in the sections, only one or two being observed in some hundreds of slides examined and the consequent absence of viable cells in the fragments inoculated. In the original tumor particle, after its implantation in plasma, many of the nuclei may have been in the resting phase when the radium was applied and hence were not seriously damaged, for, Packard (7) has shown that a resting nucleus can withstand double the exposure of a dividing one. These less influenced cells are the ones which grow in animals. The outgrowth from the original tumor must be largely composed of cells just dividing when the radium was applied or merely an ameboid wandering of cells whose nuclei are so badly damaged that they are unable to divide again and hence do not grow when planted in animals.

DISCUSSION

The interesting aspects of these experiments may be summarized as follows: First, they show a great difference in the quantities of radium required to kill a physically functionating cell, such as heart muscle, and a merely growing cell, such as is found in the connective tissues or in malignant tumors. It is probable that the nucleus of the physically functionating cell is as much damaged as that of the inactive type, but that as such cells expend their metabolic surplus in mechanical movements and not in increasing in size and in dividing, as do the connective tissue and cancer cells, the effect of the radium is not evident. We do know, however, that the heart muscle and connective tissue cell will no longer multiply in culture and that the cancer cell will no longer reproduce itself when implanted in a suitable host, so it is reasonable to assume that their death depends, to a certain extent, at least, upon the inability of the nucleus to perform the usual steps preliminary to division. In radiumized tissue we do not find multinucleated cells (in which the nuclei divide but the cytoplasm does not), but rather cells with a large spherical or lobulated nucleus, not infrequently three to four times the diameter of the nucleus of the normal cell, hence, twenty-seven to sixty-four times the volume of the norm, with cytoplasmic increase to correspond. As the surface of such a cell increases at a less rapid rate than the rate of increase of the cubic contents, it is easy to see that a point must be reached when such a cell can no longer absorb sufficient nourishment to keep it alive. If division could occur, the relative surface area would be greatly increased and this would permit a larger transfer of anabolic and katabolic products through the cell membrane. If this does not occur, the cell must die. Verworn (8) lays great stress upon these facts as a basis for the multiplication of cells. In this connection, attention may be called to the correlation between the small tissue cells of the smaller mammalia with very active metabolism, and the large cells of the cold-blooded animals.

A second fact of interest brought out in these experiments is that there is little or no difference in the radiations required to cause death of the cancer cell and of the normal cell of similar histogenesis. It has long been stated that cancer tissue is much more susceptible to the action of physical agents, than normal tissue, and unquestionably this is, roughly speaking, true. This is due to the fact that the cancer cell is a rapidly dividing, free growing cell, without well developed vascular supply, whereas the corresponding normal tissue is not dividing, except for reparative purposes, and has an ample equipment of anatomically well-formed vessels to supply its needs. If, however, we place the two types of cells under identical conditions, as they are in plasma cultures, these differences disappear and the two types are about equally susceptible to radium radiations. These facts account for the extraordinary resistance offered to radium by the slow growing squamous-cell epitheliomata.

While the experiments tend to show that the nucleus is the portion of the cell most susceptible to the radiations, the changes which are responsible for the lethal action are a mere matter of speculation.

Now that we know that ultraviolet light, the Röntgen rays, and the gamma rays from radium form a progressive series of short light waves, and that each is capable of splitting off from matter negative particles or beta rays through which they probably exert much, if not all, of their activity, it is natural to assume that such beta particles cause the lethal changes. Ultraviolet light coagulates albumin, and, whether the action takes place at a high or at a low temperature, there is practically no difference in the ultimate effect; the same is true of radium. Tissues exposed at 37°, 18°, or 3°C., all showed the same effect. The coagulation of albumin by radium requires very prolonged exposures on account of the small amount of absorption of the gamma rays by the colloid and the small amount of energy consequently set free, as compared with ultraviolet light which is strongly absorbed by protein from wave lengths of 2100 on. (9) But the comparatively coarse coagulation effects may be ruled out in the case of radium, otherwise the heart muscle preparations would be affected as easily as the growing cells. Possibly the action is of an electrical nature, for we know that the permeability of a cell membrane is altered by changing the existing state of its electrical polarization; if we decrease it, we increase permeability; if we increase it, we diminish permeability. The setting free of negatively charged particles by the gamma rays of radium occurs through all parts of the cell. It may change the cellular metabolism by direct action on all the constituents or indirectly through alterations in permeability produced in both the nuclear and cellular membranes. In addition, radiations may alter the electrical potential gradient between the cytoplasmic and nuclear areas, which according to Lillie's well known hypothesis (10) facilitates the transport of material between different regions of the cell and is responsible for some of the characteristic elements of the mitotic figures. Further studies are needed also along the ine suggested by Packard (7), who from his work on the eggs of Arbacia and Nereis believes that disturbance of intracellular ferment activities of the cell plays an important part in connection with the destructive action of radium on living protoplasm. But at present any experimental verification or rejection of these hypotheses is beyond our technical ability, even on large cells such as the eggs of marine animals, much more so on the minute and easily damaged cells of the mammalian tissues.

CONCLUSIONS

1. Radium in sufficiently large doses will so injure the nucleus of the cell as to prevent further mitosis.

2. This injury to the mitotic power of the cell does not, however, prevent a marked increase in the area of the culture due to an outwandering of cells.

3. This power of the radiumized cells to wander out from the main tissue is limited, extending through two or at most three generations.

4. When there is a marked outwandering of cells after radiumization, but no mitosis, the tissue will not grow when inoculated into mice.

5. Radium does not, therefore, kill the cells outright, as is shown by the persistence of beating in heart muscle cells, but injures the nucleus in such a manner as to prevent further division, which must eventually result in the death of the cell, if its energy is expended in growth and division and not in a purely mechanical function. The well known high resistance to radium of the cells of the central nervous system, which do not divide in adult life, is presumably correlated with the survival of the heart muscle cells after lethal exposures.

6. The stimulating effects of minimal doses of radium are shown by the profuse outwandering of the cells which occurs after sublethal exposures.

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EPITHELIOMA DEVELOPING IN PELLAGROUS DERMATITIS

SECOND REPORT

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In his report of a histological study of the skin lesions of pellagra, Gurd (1) has called attention to a degeneration of the superficial layers of the corium together with the phenomena of a mild acute inflammatory reaction in the erythematous and bullous stage of the dermatitis and a later reparative process evidenced by an increased cellularity of the corium and the presence of fibroblasts. Harris (2) in confirming an observation by Babes and Sion calls attention to peculiar homogenous metachromatic masses beneath the epithelium in the skin lesions of pellagra, which Gurd takes to be degenerated corium. These writers also call attention to a dilatation of and increase in number of the capillaries of the skin and apparently in consequence thereof to an increased proliferation of the epithelium leading to a thickening of the epidermis. Gurd says, "In the later stages, in an effort to secure a firm basement membrane, the epithelium is seen to dip down deeply into the rarified connective tissue." In comparing these changes to those described as a result of the action of the Roentgen light, this last author finds the analogy between the two so marked that "we are justified in considering that the direct agents in their production are probably similar."

From Wolbach's (3) studies upon X-ray dermatitis and carcinoma we have learned not only of the skin changes which ordinarily result from X-ray burns but have also gained valuable information concerning the development of squamous epithelioma.

The analogy between the skin lesions produced by pellagra and by the Roentgen light has not been previously carried further than the observation by Gurd that in pellagra the epithelium penetrates deeply into the diseased corium to gain a firm basement membrane, which may be taken to mean the same as the statement by Wolbach that in the changes produced by the Roentgen light it penetrates to a better nutrition. It has not until recently been shown that in pellagra a further penetration and an assumption of parasitism by the epithelium leading to the development of epithelioma, even as has resulted in X-ray dermatitis, may occur.

In a recent article I (4) reported the development of squamous epithelioma in a pellagrous skin ulcer of the thigh. This was in a negro woman who had died of a severe attack of pellagra. The epitheliomatous infiltration was purely a microscopic affair and was discovered in the routine histological post mortem examination. There was no growth manifest to the naked eye. Since no determination could be made as to whether or not any further penetration of the epithelium in this case would have taken place, the following case, which came under observation after the first report, is reported with interest in confirmation of the possibility of epithelioma developing from pellagrous dermatitis.

D. C., white, male, streetsweeper, seventy-five years of age, came to the dispensary of the Roper Hospital in Charleston March 29, 1916, with an acute vesiculo-erythematous eruption covering the skin of the backs of both hands. This he said had been present for three days and he thought was the result of sun-burn. There was no diarrhoea at the time. On March 30, 1916, his second visit to the dispensary, the dermatitis was exaggerated and it continued so until April 3, when it was improved. On April 12, his last visit in the spring, the dermatitis was still further improved, but he had developed a well marked diarrhea. A diagnosis of pellagra was made. He returned to the dispensary on September 23, at which time he complained of diarrhoea and pain in the epigastrium. The skin of the back of the left hand was then rough and scaly and somewhat red. That of the right was very red, ulcerated and moist, and surrounding the ulcerated area, which covered about one-half of the surface of the back of the hand centrally, was a fungoid irregular growth forming an interrupted circle of small, firm, red nodules. The skin in the intervals of and outside this circle was brawny, red, and rough. This marginal tumefaction was suspected of being epitheliomatous but the patient refused to have it sectioned or treated surgically. About this time a lay "cancer quack" treated the hand with an application of some sort. On November 21 he came to the dispensary complaining of weakness, and again on January 3 with indigestion.

My attention was called to him by the chief of the dispensary, Dr. E. L. Jager, about November 22, 1916. At this time the skin of the left hand had a reddish color and was somewhat rough. That of the right had healed over, the skin of the central part for an area about 1½ inches in diameter being thin, pink, newly formed, and free from hair. Surrounding this area was a zone of elevated, somewhat fungoid nodular, brawny, reddish tissué with the epiderm intact. He had no diarrhea at this time.

A few days after this I obtained a section from this circular growth. The histology of this section is similar to that of the case previously reported. The epithelium is very thick, the prickle cells and the horny layer being especially overgrown. From the epithelium of the surface there extend throughout the section, which includes some of the subcutaneous connective tissue, cords of epithelium composed of cells representing all the layers. The cells are of a large vegetative appearance, the prickle cells are abundant, and there are many hyaline "cancer bodies" and pearly bodies in the center of the cords. The outer part of the corium in this section, as in the previous case, is rarified, the degenerative change of the connective tissue cells being apparently of a lipoid nature, producing large rounded cells with a dark central nucleus and a loose reticulated cytoplasm similar to those seen occasionally in the connective tissue of chronic inflammatory processes. The blood vessels of this superficial corium are not numerous but they are plentiful toward the base of the section. In the lower part of the corium there is a progressive growth of new fibrous tissue with lymphocytic and plasma cell infiltration surrounding the cords of epithelium.

I saw the man again on January 18, 1917, at which time his hands were in about the same condition as in November, 1916. He had no diarrhea and was going about his work. He has kept the right hand covered during this time.

This report, then, is confirmatory to that of the case encountered previously (4), and brings to attention the possibility of such a result of pellagrous dermatitis in persons who have apparently recovered from the disease. It is not probable that these are the only instances of this occurrence. There have probably been others in which death from pellagra arrested the epitheliomatous development before it had become apparent and others in which the connection was not seen between previous pellagra and the subsequent appearance of an epithelioma. If the analogy between the skin changes which occur in pellagra and those which result from the action of the Roentgen light is to be considered as marked as believed by Gurd, it appears that the development of epithelioma from pellagrous dermatitis may be postponed for a much longer time than in the two cases reported.

In so far as such an application can be made, the observation of epithelioma formation in pellagrous dermatitis seems to draw the skin lesions resulting from the action of the Roentgen light, from sun-burn, including the malignant degeneration of the skin in xeroderma pigmentosum, and from pellagra closer together, and to lend support to the belief that the action of light is concerned in the development of the dermatitis in pellagra.

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TISSUE GROWTH AND TUMOR GROWTH[1]

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Tumors in general and cancers in particular are tissues growing under special conditions. A comparison between the laws determining tumor and tissue growth will deepen our knowledge of the physiology of tumors as well as of tissues. There exist similarities and analogies in the behavior of both, and there are differences either real or due to a gap in our knowledge. The differences will suggest new problems.

In this brief résumé no attempt at completeness will be made, and we will have to be content with mentioning some of the salient points. We shall first compare the causes of tumor and tissue growth, then the reactions of the host organism against tumor and tissue growth, and lastly, certain phenomena observed primarily in the tumor cells. We will have to inquire how far the last mentioned phenomena are duplicated in tissue growth.

1. Tissue growth is initiated by external factors affecting a more or less complicated system and leading to chains of reaction which may be identical, or at least similar, even in cases in which the primary causes differ. These primary causes are called "formative stimuli" in order to distinguish them from functional stimuli. It is probable that essentially both kinds of stimuli are identical and act in a similar manner. The difference in the result of both kinds of stimulation depends probably on the quantitative differences in strength and time, and on the differences in the systems on which they act.

2. The primary causes initiating tissue growth may be either

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[1] Read in a symposium on cancer before the Section of Experimental Medicine of the American Association for the Advancement of Science in New York, December 29, 1916.

physical or chemical. The physical factors are either mechanical or rays which are held back by the tissues. The chemical ones are essentially substances produced somewhere in the organism. Certain parasitic organisms which cause tissue and tumor growth act partly through chemical agencies which they produce; they may in addition exert a mechanical effect. In regard to substances produced outside of the organism and causing local tissue growth after application to certain tissues, it is doubtful how much their action is a specific chemical one and how much it is due to secondary physical factors released through injury of the tissues. Some pathologists assumed mechanical factors to be the only ones which can elicit tissue growth under experimental or pathological conditions. This view is evidently erroneous. Pathological or experimental phenomena are merely a modified interaction of phenomena occurring in the normal life of the organism. Chemical stimuli undoubtedly play a considerable part in the normal growth phenomena of the organism. This alone is presumptive evidence that they have a similar function under pathological conditions.

3. It can be shown that the stimulus brings about a series of changes in the affected tissue; these changes are partly chemical, as evidenced by increased oxidation, or physical (increased water content of the cells). They lead to a greater sensitiveness of the cells. Corresponding to their chemical and physical changes, morphological changes take place. These changes follow a definite curve. A new stimulus reaching the cell after a certain stage of this curve has been reached acts therefore on a different system and must produce different results. It has been shown that small quantities of various rays acting on various tissues may produce stimulation, while large quantities cause destruction. In conjunction with Doctor Spain we have shown that larger defects may produce a more energetic response of tissues than smaller defects; they represent a stronger formative stimulus. Successive formative stimuli lead only to a limited extent to increased reaction. Adaptive tissue changes implying an increased resistance to the stimulus are called forth. Thus a limitation in the proliferative effect is produced.

4. If a strong growth stimulus affects tissues under otherwise unfavorable conditions, as for instance at a place where the supply of food and oxygen is diminished, an abnormal response of the tissues takes place (irregular mitoses, amitotic nuclear divisions, formation of giant cells and syncytia). If the conditions of nourishment become still more unfavorable, necrosis follows. Quite generally growth stimuli more or less inhibit the full morphological differentiation and correspondingly the full metabolic correlative activity of tissues. This effect finds expression in a simpler structure of various growing tissues.

The facts mentioned under No. 3 and No. 4 apply on the whole equally to normal tissues and tumors, but they are usually more marked in the case of tumors, especially cancers, because here either the growth stimuli are stronger or the conditions of growth are more irregular and therefore more unfavorable as far as nourishment is concerned.

5. These same physical factors, the formative stimuli for tissue growth, when acting over a long period of time, lead in certain individuals to the production of tumors or cancers. It has been possible to produce cancers experimentally through the application of physical or physico-chemical factors (P. Marie, Fibiger). These experimental facts correspond to observations in human cancer. Apparently the transformation from normal tissue to cancer is a gradual one, passing through several intermediate steps. However, if we work with either normal tissues or cancers or intermediate formations (regenerating tissue, inflammatory new-formation, benign tumors), in most cases they retain their original characteristics in a fairly constant manner during the period of experimentation.

6. Formative stimuli of a mechanical nature act on certain cancers as they do on normal tissues. Cuts, incomplete extirpation, pulling threads through tumors that have become stationary, transplantation of tumors, act as stimulants and increase the energy of growth.

7. Chemical formative stimuli undoubtedly play a considerable rôle in the growth of normal tissues. From the data which are accumulating, it may be possible in a provisional way to

classify these chemical stimuli into (*a*) general stimuli, applying indiscriminately to a large number of tissues; (*b*) specific stimuli acting only on specific tissues. Chemical factors of the former kind are probably operative in *young* organisms, in contradistinction to old ones, and during pregnancy. In the last named condition not only factors favoring growth are apparently at work, but also factors antagonistic to growth. The balancing between these two forces seems to lead to a different result in different species. Thus in the rat, pregnancy seems to favor growth of embryonal tissues under certain conditions; in the mouse, it is unfavorable to such growth. Tumor growth is affected in a way similar to normal tissues by the chemical conditions prevailing in young and old organisms respectively. The difference in the growth of certain organs in young and old animals seems to depend on substances circulating in the body fluids. Analogous substances seem to hasten or to delay growth phenomena associated with metamorphosis in amphibia. There is also some indication that during pregnancy spontaneous tumors may assume a marked increase in size in the rat, while in the mouse, pregnancy is an unfavorable factor, especially for the growth of transplanted tumors. In future it will be necessary to distinguish more sharply than has been done in the past between the effect of pregnancy on the growth of spontaneous and of transplanted tumors.

The corpus luteum, which stands in specific relation to the wall of the uterus and perhaps to the mammary gland, is an example of the second kind of growth substances. There can be no doubt that these specific growth substances play an important rôle also in the transformation of normal into cancerous tissues. This has been proven experimentally by the writer in the case of the cancer of the mammary gland in mice. By extirpation of the ovaries at the time maturity has been reached, the spontaneous development of cancer of the breast, which is so common in certain strains of mice, can be almost altogether prevented. We may assume that other specific correlative growth substances play a similar part in the cancerous transformation of other tissues.

There is another class of growth substances, namely those which prevent growth and the absence of which promotes growth. We might call those: "negative growth substances." We might include iodine in this category which, according to Marine, prevents compensatory hypertrophy of the thyroid. In a similar manner, according to Raymond Pearl, calcium seems to counteract certain of the actions of the corpus luteum substances; but whether it inhibits the growth processes called forth by the corpus luteum, has not yet been investigated.[2] There is, however, some indication that calcium inhibits somewhat the growth of inoculated tumors, although it is not certain that the evidence in this direction is conclusive.

8. There are cases in which a chemical and physical stimulus must coöperate in order to induce considerable tissue growth. The formation of the maternal placenta is an example of this kind of growth. A definite relationship exists in this case between the chemical and physical stimulus. The chemical stimulus has to precede the physical (mechanical) stimulus. It sensitizes the tissues to the action of the physical stimulus.

However, in order to obtain the fullest possible growth reaction, the chemical stimulus must continue to act after the onset of the physical reaction chain. A similar combination between chemical and physical stimuli seems to play a part in plant growth, as has recently been discovered by Haberlandt. It seems to be of significance also in the case of certain plant cells, which in some respects correspond to the benign tumors of animals. It is probable that it plays a considerable part in the etiology of animal tumors, but definite data are as yet lacking in this respect.

9. While etiological factors established for tissue growth thus point the way for a search for analogous conditions in the case of tumor growth, the converse condition also exists. Certain factors established in the etiology of tumor growth have not yet found their analogue in the case of tissue growth. We refer especially to the great part heredity plays in tumors. It has to be added as an internal factor to the external factors, which

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[2] We are carrying out such experiments at the present time.

we mentioned above. Analogous hereditary conditions in the case of normal tissue growth are not known. In conjunction with Dr. Addison, the writer has, however, established the fact that homologous tissues in different species possess normally a very different degree of proliferative activity, which is hereditarily fixed. It is also possible that exceptional instances in which experimentally produced tissue growth took an unusual course in certain individuals, may be due to hereditary peculiarities in these animals. I refer to the observations of Askanazy, who found twice that experimentally produced embryomata became malignant, and to an observation of Stieve, who observed that inflammatory proliferation around foreign body giant cells assumed in one case certain characteristics of a malignant tumor. Similarly the writer found with Dr. Fleisher that in contradistinction to others, certain benign tumors are transplantable. As to the character of the hereditarily fixed factors, we may perhaps suggest that hereditarily transmitted differences in chemical sensitizations, similar to those determining the growth in the uterine mucosa, may be responsible for this phenomenon.

10. The effect of formative stimuli greatly varies in accordance with the system on which they act. It is very great in the case of the egg, which is ready to respond to the proper stimuli with an extraordinary reaction chain consisting of a combination of cell multiplication and differentiation. It is otherwise in the case of differentiated tissues, where the response is relatively slight or is lacking altogether after a certain degree of differentiation has been reached. In some cases at least this differentiation can be shown to be due to unfavorable conditions of nourishment. While in the case of the tissues of the differentiated organism a certain relation exists between the faculty on the part of normal tissues to respond to growth stimuli, and the capability of these tissues to cancerous transformation—a parallelism which can be readily understood on the basis of what we said above concerning the part of growth stimuli in the origin of tumors—it is very interesting to note that the direct cancerous transformation of tissues within the embryo must be an extremely rare occurrence, although later, after the completion of the em-

bryonic development, in certain cases tumors may be found which developed on the basis of embryonal malformations; or other tumors may originate in a developed organism through parthenogenetic development of the ovum. Cancer means growth without differentiation and in the embryo growth is linked with differentiation. In a similar manner, from what information we have at present, we must assume that in invertebrates likewise cancer must be extremely rare. These facts are in apparent contradiction to the fact mentioned above, that only tissues capable of growth become cancerous, and most differentiated tissues do not become cancerous. However, the contradiction is only apparent. Cancer originates in tissues capable of growth, provided they are parts of a rigid whole in which the growth stimulus cannot lead to regulatory growth processes. Where, therefore, organisms readily respond to growth stimuli with budding and various other kinds of organic reproduction, or with far going regenerative restitution—a capability much more pronounced in the earlier phylogenetic and ontogenetic stages—cancer must be rare. This does not exclude the possibility that it may occur, especially if we consider the fact that in different invertebrates great variability exists as to the power of restitutive processes. Furthermore, cancerous transformation of tissues seems to be much more common in organisms with marked chemical correlation of different parts of the whole organism—a chemical correlation which we have reason to believe is absent or much less rigid at early ontogenetic or phylogenetic stages.

11. There exists a series of observations which point to the conclusion that one tissue may exert a localized effect on a neighboring tissue. Thus in certain cases we find the connective tissue of the mucosa directly underneath the epithelium more cellular than at a distance from the surface epithelium. The actively secreting or proliferating mammary gland is surrounded by a stroma rich in cells and poor in fibrous tissue; the resting gland on the other hand has a dense fibrous stroma. Tissue transplanted from a strange individual calls forth a more marked production of fibrous tissue than tissue transplanted from the

same individual. Contact with bone seems to stimulate periosteum to the production of bone, which is not produced by periosteum after transplantation into muscle. Certain parts of the optic cup transplanted underneath the epidermis call forth the production of lens or cornea from the epithelium of the skin. The writer has observed that cell proliferation in the granulosa of the graafian follicles may be especially marked around the ovum. The influence of the ovum on the proliferation of the granulosa has been recently established in our laboratory through the quantitative investigations of Newman Walsh. We find similar phenomena in the case of plants. Haberlandt has shown that from the leptom of certain plants substances diffuse into neighboring tissues and produce cell multiplication. There exists a similar observation in cancer; carcinoma may cause the development of sarcoma in the surrounding connective tissue stroma. Whatever the ultimate interpretation of this phenomenon may be, it is well to keep in mind the analogies of this process with certain occurrences in the growth of normal tissues.

12. If we leave now the discussion of the origin of cancer and consider the fully established cancer, we may state that cancer is tissue growth in which a chemical stimulus, presumably not very different from those substances which play a rôle in normal tissue growth, is constantly at work producing those physico-chemical changes which accompany all growth. The etiological problem of cancer, which remains still to be solved, concerns the question whether this chemical stimulus has to be transmitted to the tissue cells by an outside agency in close proximity to the tumor cells or whether long continued stimulation of tissues ultimately leads to such a change in the cell mechanism that the cell itself is enabled to produce continuously the substance calling forth propagation. Both possibilities may be realized in different cases. In fowl sarcoma Rous succeeded in separating from the living cells an agency producing sarcoma. Nothing definite is known about its character. According to E. F. Smith, certain plant tumors bearing some resemblance to animal cancer are caused by a chemical agent continuously produced by Bacterium tumefaciens; while in the common galls chemical

agencies produced by parasites cause a growth process that ultimately comes to a standstill, in the case of the so-called crown gall the growth caused by the bacterium seems to be continuous. According to Friedemann, the same Bacterium tumefaciens or nearly related organisms cause in man inflammatory processes, namely meningitis and arthritis, another demonstration of the importance of the system on which a stimulus acts in the determination of the final result.

13. In this connection we wish once more to come back to the fact mentioned above, that internal secretion may be responsible for the production of cancer. It was known before that long continued stimulation may cause cancer. In those cases, however, ulceration was almost invariably connected with the process of stimulation, and it was pointed out that the chronic ulceration rather than the stimulation was the cause of the development of cancer, this ulceration presenting perhaps to cancer producing parasites a favorable opportunity to gain entrance into the tissue.

In the case of the chemical stimulation of the mammary gland eventuating in cancer, no ulceration precedes cancer and still the growth stimulus is the active factor in the production of cancer. We may therefore conclude that it is the direct growth stimulus and not a secondary effect of ulceration which causes cancer in these cases.

14. The reactions of a host organism against induced tissue and tumor growth are very similar, as the following comparison will show: (*a*) In the organism in which a tumor originated usually no reaction takes place against tumor cells after transplantation. After autotransplantation of a piece of normal tissue it may apparently live indefinitely. This holds good at least in the case of certain tissues. (*b*) After homoiotransplantation of normal tissues, as the writer and his collaborators, Myers and Hesselberg, have shown, the tissues die as a result of the attack by lymphocytes and through the influence of fibroblasts of the host, which produce dense fibrous tissues which may strangulate the foreign cells. There is a possibility that the strange body fluids may also directly interfere with the metab-

olism of certain transplanted tissues to such an extent as to injure them severely. It has been likewise demonstrated that in the case of tumors against which an immunity becomes established, lymphocytes, sometimes in conjunction with other leucocytes, play an important part in the destruction of tumor tissue (Burgess and Tyzzer, Baeslak, Rous, J. B. Murphy, and others). Russell as well as Burgess noticed in addition the production of a dense stroma after transplantation of tumors into nonsusceptible mice, without, however, apparently insisting on the significance of this phenomenon. In addition Russell in the case of tumors, and P. Rous in the case of normal tissues, believe that immunization results in the development of some factor that delays or diminishes the ingrowth of stroma into the transplant. (*c*) After heterotransplantation normal tissues as well as tumors die after a transitory period of life or even of growth.

How far in these various cases primary incompatibility between tissue and body fluids of the host, how far secondary immunization called forth by the introduction of the foreign tissue or tumor play a part, is uncertain at present. In the case of homoiotransplantation of tumors it has been demonstrated that active immunization plays a great rôle in the defensive reactions. This, however, does not exclude the possibility of the simultaneous existence of primary incompatibilities between tumor cells and host body fluids. In the case of normal tissues only a very limited number of experiments exist which point to the development of immune substances as a result of repeated inoculation with normal tissues (Fichera, Schoene, P. Rous).

Of the attempts to unite the various facts into a consistent theory of tumor and tissue immunity only two recent ones may be mentioned; (*a*) without concerning himself with the growth of normal tissues, E. E. Tyzzer explains the immunity against tumor growth in a manner somewhat related to von Dungern's and Coca's interpretation as due to a local anaphylactic reaction. According to Tyzzer some substance is given off by the tumor cells which combines with the body fluid of the host. This new product injures the tissue surrounding the transplanted tumor. Tyzzer emphasizes the conclusion that the surrounding tissue is more sensitive, more readily injured by such a substance than the tumor cells which are held to be more resistant. (*b*) Our interpretation, which represents a further development of conclusions published in 1907, is as follows: The mutual chemical incompatibility of the body fluids of one individual and the tissues of another, which was particularly striking after homoiotransplantation of pigmented skin, leads to changes in the metabolism of the tissues resulting in the production of homoio- and heterotoxins, which if they do not exceed a certain quantity or degree of toxicity, disturb to some extent the normal functions and metabolism of the transplanted tissues without, however, seriously interfering with their life. But the abnormal products formed attract the lymphocytes and in certain cases perhaps also other leucocytes and alter the reaction of the fibroblasts which latter are induced to produce dense fibrous tissue. If the poisons become more active, they may directly injure the transplanted tissues to such an extent that growth and life become impossible. In a similar way we believe in so-called chronic inflammatory processes of various organs, a changed metabolism of the cells and perhaps also poisons produced by microörganisms may induce fibroblasts to form fibrous bands and attract lymphocytes, thus leading to processes of cirrhosis. In an analogous manner in the case of tumor immunity substances produced as a result of immunization and secreted into the circulation, alter the metabolism of the tumor cells which now attract lymphocytes and change the activity of fibroblasts in a way corresponding to that which we described after transplantation of normal tissues into a strange host.

This theory correlated the immunity against tumor growth with that of the growth of normal tissues and also with the immunity against certain substances and non-growing foreign cells. We have according to our theory also in the former case to deal with the production of immune substances which, however, after homoiotransplantation, are usually not such that they directly destroy the foreign tissues but merely lead to an alteration of their metabolism and to the production of substances which change the behavior of the host cells. We no longer need to assume a primary tissue alteration following the homoiotransplantation as E. E. Tyzzer did.[3] Furthermore, our theory not only explains tumor immunity as an inflammatory reaction, but conversely throws light on the phenomena of chronic inflammation through the analysis of tumor immunity; it correlates tumor immunity and inflammatory processes with other phenomena apparently of an entirely different character, but in reality, according to our opinion, nearly related to the former, namely the variations in the behavior of the stroma of certain glands and also the differences in the character in the connective tissue of the mucosa nearby and at a distance from the epithelium.

The resistance against transplanted tumors acquired either spontaneously or through previous inoculation with normal or tumor tissues of the same species partakes somewhat of both the typical immune as well as the anaphylactic reaction. It resembles an anaphylactic reaction in so far as the presence of tumor tissue in an immunized (sensitized) animal calls forth secondarily the production of substances which directly act on the cells of the host, changing their activity. The primary substance, however, acts on foreign tumor or tissue cells and modifies their metabolism. It resembles the typical process of immunity inasmuch as the ensuing reaction does not cause an injury of the host, but of the introduced foreign tissue or tumor.

We may assume that the large majority of the cells of an individual of a certain species have a certain chemical group in common, which we may call the individuality differential and which causes characteristic differences between the various individuals of the same species. In addition we have family, race or strain differentials common to members of certain families, races or strains. There may be still finer gradations through which brothers and sisters are differentiated. There are, furthermore, species differentials which members of a certain species have in common and through which they differ from other species. Cancer cells may possess all of these differentials just as ordinary tissue cells, but it is probable that a certain number of cancers lack certain kinds of these differentials. And not infrequently the cancers belonging to certain classes of tumor seem to be similar in this respect. Thus the lymphosarcoma of dogs lacks the majority of those differentials. In a tumor of a Japanese mouse which we found in 1905, and in a similar one found recently by Tyzzer, the individuality differentials were apparently entirely lacking. But the majority of cancers possess these individuality differentials in a manner similar to normal cells. The loss of these differentials is therefore not an essential character of cancer and cannot enter into the etiology of cancer.

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[3] In a more recent publication Tyzzer adduces some experimental evidence confirmatory of our view and accordingly modifies somewhat his views which now approach very closely our interpretation.

Great growth energy on the part of tissues and tumors seems to be able to overcome to some extent the barrier against proliferation set up by these differentials. Thus rapidly growing embryonic and tumor tissue overcomes at least temporarily these barriers, but it is probable that in embryonic as well as in phylogenetically lower adult tissues some of the differentials are as yet not fully developed. How far these differentials act alone or in combination with constituents of the body fluids of the host as primary homoio- or heterotoxins, how far they act only through the production of secondary immune toxins, is as yet not fully determined. But it is certain that in all these reactions no essential difference exists between tumors and normal tissues.

We now wish to consider very briefly some interesting features in the growth of tumors which depend mainly on the character of the tumor cells and inquire how far analogies to these phenomena can be found elsewhere under normal conditions.

15. In addition to the increase in proliferative power, the ability to infiltrate and substitute neighboring tissues is characteristic of cancers. The same property is normally found in the chorionic cells infiltrating the wall of the uterus or the ovary in case of parthenogenetic development of the ovum. A similar invasion of neighboring tissue, according to the observations of

the writer, takes place in guinea-pigs, if through transplantation nonpigmented epidermis is placed in direct contact with pigmented epidermis. Under those conditions constituents of the pigmented epidermis begin to invade and substitute the nonpigmented epidermis. An analogous phenomenon has recently been observed by Weigel in the case of the epidermis of amphibia.

16. The resistance of tumors to physical and chemical agencies has been determined in various varieties of tumors, especially in sarcoma of the rat and carcinoma of the mouse. In both cases the degree of resistance was found to be very similar to that of normal tissues. The agent producing sarcoma in fowls is more resistant than tumor cells and so is the sarcoma found at the external genitalia of dogs. This raises the question whether also in the case of the latter an agent dissociable from the tumor cells proper is responsible for the growth in certain cases. It is, however, to be noted that after transplantation of the dog sarcoma into other dogs the peripheral tumor cells survive and give origin to the new growth.

17. As has been shown by the writer, the growth energy of tumor cells can be experimentally increased and decreased. Various explanations have been given for these phenomena, but we believe that they are due to a direct action of physical and chemical agencies on the cells. According to this interpretation, the experimental increase in growth energy is comparable to the increase in growth energy we find in normal tissues as a result of regenerative stimuli. It has, however, not been possible so far to increase the growth energy of normal cells through *serial* transplantation within the living organism, a result commonly obtained after *serial* transplantation of tumors. However after serial transplantation of connective tissue *in vitro* a similar increase in growth energy seems to take place. A somewhat analogous increase in virulence after serial inoculation has been observed in the case of certain microörganisms.

The decrease in growth energy of tumors exposed to injurious agencies finds a parallel in the effect of certain injurious agencies on certain plants and in the action of radium on embryonic tissues. It has likewise been observed in the case of microörganisms exposed to such weakening agencies.

18. In experiments by Fleisher and the writer it has been possible to demonstrate in cancer an active immunization of the tumor cells against chemical agencies inhibiting their growth energy. This immunity is transmitted to a number of successive cell generations. It is probable that a similar immunity may also be acquired against the action of certain physical agencies as for instance radium or roentgen rays. The immunization of epidermal cells against the repeated application of cold or heat and radium rays, described by Werner, represents probably a parallel phenomenon in the case of normal tissues, although an inheritance of this immunity to following cell generations has not been demonstrated in this case. The experimental production of strains of trypanosomes resistant toward certain specific agencies is a similar occurrence in the case of unicellular organisms.

In our experiments we could show that an actual immunization of tumor cells takes place and not merely a selection of cells possessing a greater natural resistance to the injurious agency. There are on record some other observations of a somewhat similar character which however at present may be explained either on the basis of cell immunization and gradual adaptation or on the basis of a selection of cells naturally better adapted to certain environmental conditions. Thus Koenigsfeld states that if metastases are used for inoculation, the resultant tumors are more liable to produce metastases than if the primary tumors are employed. Ebeling finds that tumors which once have been successfully transplanted into the brain—a result which at first is obtained only in a relatively small number of cases—can subsequently be propagated through transplantation into the brain with much greater readiness.

19. Bashford, Murray and their collaborators in starting from a small piece of tumor and carrying out long series of tumor transplantations, in many cases observed the development of substrains from the apparently homogeneous tumor mass. The characters of such substrains, which concerned morphological as well as functional variations, remained more or less constant throughout consecutive transplantations. Do we have in these cases to deal with a selection of genotypes within the tumor

originally representing a colony of several mixed strains, or do we have to deal with mutations or variations leading to the formation of substrains, such as they have been recently described, especially by Jennings in the case of protozoa, and by various investigators in the case of bacteria? At present we are unable to answer this question. A similar phenomenon has to our knowledge not been observed in the case of normal tissues.

20. The long continued transplantation of tumor suggested first the potential immortality of somatic cells which must apply equally, to tumor and to normal tissue cells, the former being merely tissue cells living under particular conditions.

This necessarily incomplete comparison between phenomena observed in tumor and tissue growth may suggest that a study of tissue growth may not only assist in the interpretation of tumor growth, but that conversely, the analysis of tumor growth may help to lay the foundation for a physiology of tissue growth.

Furthermore, this comparison leads to the conclusion that the majority, and ultimately perhaps all, of the phenomena observed in tumor growth find a parallel in the growth of normal tissues under special conditions.

THE SIGNIFICANCE OF THE LYMPHOCYTE IN IMMUNITY TO CANCER

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Many writers have described the presence of the mononuclear lymphocyte in the reaction zone around a foreign body or a transplanted tumor graft. Da Fano (1), among the first, pointed to the cellular reaction of the tissues of mice to implanted tumor grafts; and because lymphoid and plasma cells are constantly associated with degenerating and receding tumors (which cells persist until the graft becomes absorbed), he assumed that these lymphoid cells play perhaps an important part in tumor immunity.

Similar results were reported by Tyzzer (2). In control non-immune mice, an inflammatory reaction around the implanted tissue occurs, which generally disappears within seventy-two hours. In resistant mice, however, the reaction persists and the surrounding tissue becomes infiltrated with a cellular exudate, containing various types of leucocytes, polynuclear and phagocytic, endothelial cells, lymphoid cells, plasma cells, and a good many eosinophiles. Inoculation of dead tumor cells, however, is accompanied neither by this lymphoid cell infiltration, nor by the development of tumor immunity.

Baeslack (3) attempted to determine whether growing or receding tumors had any influence on the ratio of white blood cells. In mice with spontaneous and actively growing tumors, he described a constantly high polynuclear leucocytosis and in those with receding or unsuccessful transplants, a marked diminution of leucocytes and a corresponding increase of mononuclear lymphocytes. This inverse ratio of leucocytes in the circulating blood led him to suggest that the small mononculear lymphocytes might stand in casual relationship to immunity, though he arrived at no definite conclusion.

Kenneth Taylor (4), to the contrary, in a recent publication, mentioned a naturally high lymphocytic count in normal albino rats. The differential count averaged 30 per cent small, and 14 per cent large mononuclear lymphocytes in a total of 19,000 to 30,000 white blood cells per cubic millimeter. Moreover, during the active growth of the tumor, no marked change of the blood picture was noticeable, except perhaps a slight increase of the small mononuclear lymphocyte. Only when the tumor became ulcerated was there a marked polynuclear leucocytosis of 50,000 and upwards. My own observations confirm this slight, but probably in no way characteristic, reaction of the cellular elements of the blood in tumor-bearing animals.

Rous (5) at first, and later Murphy (6) and Morton (7) (8), regarded the degree of lymphocytic reaction in the zone around the implanted graft as an index to immunity. Murphy and Morton's reports have evoked great interest in as much as they have attempted to gauge the importance of it by experimental methods. A marked depletion of the lymphoid elements in the blood, produced by repeated doses of X-ray, they asserted is accompanied by loss of natural or induced resistance to the growth of tumor inoculation, i.e., a potentially immune animal will become susceptible to cancer implantation, when deprived of its defensive mechanism against the activity of the implanted graft. They also emphasized the importance of this lymphoid reaction in resistance to experimental tuberculosis, as well as to transplantable cancer. Contrary to this, Kessel and Sittenfield (9) reported that the administration of repeated doses of X-ray to guinea pigs with experimentally induced tuberculosis, was followed by healing of the tubercles by fibrosis; whereas no increased amount of lymphocytic reaction around the lesion was to be demonstrated. In spite of the absence of lymphoid activity, the resistance of the animals, judged by the prolongation of life, appeared to be enhanced.

According to Murphy and Morton, the small mononuclear lymphocyte in the reaction zone, or in the circulating blood, constitutes the essential factor in the immunity mechanism. When by a previous exposure to X-ray the lymphoid cells in the blood are destroyed, the resistant state is so completely abolished that a refractory animal is thus rendered susceptible. On the other hand, an artificial immunity to transplantable cancer can be induced by stimulating doses of X-ray, so administered as to bring about a definite lymphoid crisis in the blood. For instance, when tumor-bearing mice, whose tumors were temporarily removed, are subjected to a small or stimulating dose of X-ray, followed immediately by reinoculation with their own tumors, a refractory state to tumor growth is brought about, evidenced by the very small percentage of takes. On the other hand, when the same dose of X-ray is administered to the excised tumor of the mouse and again reinoculated into the former tumor-bearing animal, no resistance can be induced.

Contamin (10), however, stated that if cancer cells are irradiated by X-ray or radium for a short time, and afterwards inoculated into mice, a considerable degree of immunity is obtained; too long an exposure however, to X-ray, destroys this immunity-conferring power of the cells.

Wedd, Morson and Russ (11), on the other hand, reported that although no immunity is produced when normal and radiated pieces of tumor are simultaneously inoculated, the inoculation of radiated tumors alone does produce a slight resistance to later implantation of normal tumors.

The many conflicting views concerning the importance of lymphoid reaction in the circulating blood or around the graft, made it desirable to investigate the relationship of the small mononuclear lymphocytes to immunity in cancer, and the hypothesis, therefore, was subjected to a series of experimental tests which were arranged as follows:

1. A hyper-lymphocytosis was induced in normal rats by the subcutaneous injection of pilocarpine, and the rats then inoculated with Flexner-Jobling rat carcinoma.
2. Intravenous injection of leucocytic cream obtained from X-rayed rats, rich in lymphocytes were made into normal ani-

mals and followed by inoculation with Flexner-Jobling rat carcinoma.

3. *Nullers* whose absolute immunity had been tested by repeated tumor inoculation, were subjected to large and repeated doses of X-ray producing a marked reduction of lymphocytes, and subsequently inoculated with Flexner-Jobling carcinoma.

Experiment 1. Artificial lymphocytosis was induced in normal rats by injecting them subcutaneously with pilocarpine on alternate days. It was observed that the lymphoid cells in the blood did not increase very markedly until at least thirty-six hours after the injection, the optimum reaction occurring at the end of forty-eight hours and then gradually declining. In some instances, the small mononuclears rose from 12 per cent to 55 per cent, the total white blood count ranging from 18,000 to 34,000 per cubic millimeter. The total white blood count and differential were also taken in normal rats, and in these the small lymphoid cells were found to vary from 9 to 16 per cent although the range of variation was less in the control animal. After five injections of 0.0003 gram pilocarpine, when this artificially produced lymphocytosis was at its highest, Flexner-Jobling rat carcinoma was inoculated, and the pilocarpine injections continued until a nodule appeared, generally on the twelfth to the fourteenth day. In some of the animals the injections were discontinued at the time of transplantation. At this time all the injected rats showed a very high lymphoid count, ranging from 35 to 52 per cent. Twenty-nine rats received pilocarpine and were inoculated, together with 23 controls. In the injected rats successful growth resulted in 16, or 55 per cent, and in the controls 13, or 57 per cent. The tumors were about of the same consistence, perhaps in some they were slightly smaller, and in about 50 per cent after the twenty-fifth to the thirtieth day, as is frequently observed in Flexner-Jobling carcinoma, some of the tumors necrosed and degenerated.

	NUMBER OF RATS	LEUCOCYTES BEFORE INOCULATION	SMALL MONONUCLEARS	NUMBER OF INJECTIONS BEFORE INOCULATION	NUMBER OF INJECTIONS AFTER INOCULATION	SMALL MONONUCLEARS AFTER INJECTION	PER CENT OF TUMOR GROWTH
			per cent			*per cent*	
Experiment	29	18–34,000	12–55	5	5	35–52	55
Control	23	8–14,000	9–16				57

Experiment 2. Normal rats were exposed to stimulating doses of X-ray by means of the Coolidge tube. The administered dose was through a 2 mm. aluminium filter, 25 cm. focal distance, 2.5 milliamperes for two to three minute penetration by Bauer scale, 0.5 mm. lead absorption, equaling 2 to 4 Kienböck units. This was repeated on alternate days, until the lymphoid cells were increased to 20 to 30 per cent of a total of 12,000 to 18,000 white blood cells. These animals were then anaesthetized lightly, and the thorax opened in order to draw the blood from the heart. This blood was mixed with 0.2 per cent citrate of soda solution and centrifuged for fifteen minutes. The creamy layer on top consisted chiefly of polymorphonuclear and mononuclear leucocytes and some red cells. The leucocytic fluid thus obtained was diluted in 1 cc. Ringer's solution and injected into the jugular vein of normal rats. Generally after two days they were inoculated with Flexner-Jobling rat carcinoma; in some, however, the inoculation had been made two to four days previous. In nearly all the experimental animals the small mononuclear lymphocyte had risen to 20 to 25 per cent. Out of 40 injected and inoculated rats, 23, or 57 per cent had tumor growth, and of the 30 normal rats which were inoculated as controls, 17 showed tumors, i.e., about the same percentage of takes.

	NUMBER OF RATS	PER CENT OF SMALL MONONUCLEARS AFTER INTRAVENOUS INJECTION OF LEUC. CREAM	DAY OF INOCULATION AFTER INTRAVENOUS INJECTION	PER CENT OF TUMOR GROWTH
Experiment	40	20–25	2-4	$57\frac{1}{2}$
Controls....................	30			$56\frac{2}{3}$

In the third set of experiments, 19 immune rats which had previously been inoculated three times, and some even four times, to test their absolute resistance to Flexner-Jobling tumor, were subjected to large doses of X-ray every third day for a period of fifteen days, and then inoculated with the same tumor. These large doses of penetrating ray were administered to cause a diminution of the lymphoid element in the blood of 4 to 7 per cent. In only 2 rats did a small nodule appear, and this was absorbed on the fifteenth and seventeenth day respectively. Of the 10 controls 6 showed tumor growth.

	NUMBER OF RATS	TIMES OF REINOCULATION	PER CENT OF SMALL MONO-NUCLEARS AFTER LARGE DOSES OF X RAY	PER CENT OF TUMOR GROWTH UPON INOCULATION
Nullers	19	3–4	4–7	10*
Control	10			60

* Disappeared after seventeen days.

These facts just cited would seem significant, considering that a lymphocytosis of such proportion as that produced in normal animals throughout a period of fifteen days preceding the implantation of a growth, and then for fifteen days subsequent, did not exert the least influence upon the cancer growth, either to protect or to inhibit it. On the reverse side, a depletion of these cells by repeated doses of X-ray in the refractory animals did not alter their state of resistance in the slightest, judging from the ineffectiveness of an inoculation of tumor graft, to which they were resistant.

Just what the defensive and protective mechanism is, seems still in doubt, since the increase or destruction of the lymphoid element in the blood does not give any evidence of immunity nor susceptibility.

Uhlenhuth (12) and others have shown that a tumor most carefully and thoroughly excised will not recur, while incomplete removal of the tumor will predispose to recurrence. These factors must be taken into consideration, in addition to the influence of X-ray upon the lymphoid cells.

CONCLUSIONS

A high degree of lymphocytosis, caused either by subcutaneous injections of pilocarpin or by the intravenous introduction of leucocytic cream from rats that had received stimulating doses of X-ray, affords neither a protective nor a defensive mechanism against tumor inoculation.

Naturally immune rats, which had had their immunity tested by three or four inoculations of Flexner-Jobling rat carcinoma and were then subjected to repeated doses of X-ray, causing in them a very low lymphoid count in the blood, remained refractory to further inoculation of the Flexner-Jobling tumor.

Neither increase nor reduction of the lymphoid elements in the blood had any influence upon either resistance or susceptibility to tumor growth.

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STUDIES IN THE INFLUENCE OF VARIOUS FACTORS IN NUTRITION UPON THE GROWTH OF EXPERIMENTAL TUMORS. I

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The problem of cancer is but one aspect of the broader problem of growth. Just as the young organism is distinguished from the adult by its power to grow, so the cells of the tumor differ primarily from those of the host in their capacity for rapid, and apparently unlimited, increase. Unless proved to the contrary, it must be assumed that the same agencies which are responsible for growth in the young organism are also concerned in the growth of the tumor. Whether the various factors concerned are of equal importance in the two processes, whether each bears the same relation to growth under the two conditions, is at least partly open to experimental investigation at the present time.

Within recent years, the researches of Hopkins, Mendel and Osborne, McCollum, and Funk, together with a number of other investigators, have opened a new era in the science of nutrition. Diets which were formerly believed to be sufficient to maintain life and growth have been found to be wholly inadequate to do either. According to recent conceptions, we may regard diets as "deficient" in any one or more of four ways. Firstly, a diet may be deficient in protein, in inorganic salts, or in water; secondly, it may be deficient in content of calories, i.e., it may not yield enough energy for the requirements of the organism, through a quantitative deficiency in carbohydrates, or fats, or both. Thirdly, it may be deficient in certain fundamentally necessary amino acids, which the organism cannot synthesize. An example of a diet of this type would be one properly balanced in regard to protein, fat, carbohydrate, inorganic salts, accessories, and water, from a quantitative standpoint, but in which the sole source of nitrogen is gelatin or zein. Certain amino acids which the organism cannot synthesize are lacking in such a diet, and the animal will perish after a more or less brief period if they are not furnished. Lastly, the diet may be complete in regard to the essential ordinary food-stuffs while lacking in the so-called accessory food substances (the "water-soluble A, and the fat-soluble B" of McCollum, the "vitamines" of Funk). If such "accessory" food materials are not supplied, the death of the organism will surely ensue. No one has succeeded in maintaining the life of an animal over a reasonably long period upon any diet of chemically purified food-stuffs.

It has been planned in this laboratory to undertake a systematic study of the relationships between the growth of normal and of tumor tissue, with especial reference to the fundamental factors which are at present known to influence normal growth in young animals.

Studies along this line have already been reported by certain investigators, both from this laboratory and from others. These will be referred to at the points where our work touches that of previous investigators.

The present paper is preliminary in nature, but we feel that its publication will be justified if it serves only to call attention to the inherent difficulties in this field of investigation. In addition to this, however, we hope to present some facts which are of interest in relation to the general question of nutrition, and which may have their proper application to the problem of cancer.

The first question which we have taken for study is whether tumor cells, like somatic cells, are dependent upon a supply of vitamines from outside sources, or whether tumor cells possess a power of synthesis of vitamines. If this latter supposition should be correct, then we should have a fundamental difference between tumor and body cells, a difference as great in this respect as between plant and animals cells. The former of these synthesize vitamines; the latter have never been shown to do so. If tumor cells possess the power of vitamine synthesis, then they have indeed a unique and tremendous advantage over ordinary tissue cells. Obviously this question is an important one, and worthy of detailed study. Investigations planned to deal with this problem have already been reported. Funk (1) found a higher percentage of "takes" and better growth for the Rous sarcoma when inoculated into chickens on normal diets than when the chickens were fed on unpolished rice with or without addition to this diet of yeast (rich in vitamines) or of sarcoma extract. With chickens on polished rice (vitamine-free) Funk failed to get "takes" with the Rous sarcoma. From the vitamine standpoint these results are somewhat contradictory, since the tumor grew better in the birds upon a "normal" diet than in one known to be rich in vitamines (unpolished rice plus yeast). Chickens are not, however, well adapted for an experiment investigating the relation of vitamines to tumor growth, since the agencies determining normal growth in chickens have not been at all adequately studied. Funk (2) has reported investigations along this line, but the results so far do little more than show that the growth-controlling factors in chickens are quite different from those in the mammals so far studied. Indeed from Funk's findings we are convinced that at present no study having any real significance in relation to the question of whether tumor growth differs essentially from normal growth can be successfully carried out upon chickens.

Rous (3), and Sweet, Corson-White, and Saxon (4) have studied the effects of underfeeding and of certain deficient diets upon the growth of tumors in rats and mice. The diets they employed were probably deficient only in one or more specific amino acids. Under such conditions it seems probable that the tumor tissue may take the animo acid necessary for its own nutrition from the circulating proteins in the blood, where it probably is present in abundance so long as the animal remains alive. The results of Rous, and of Sweet, Corson-White, and Saxon are not, however, directly related to the question which we are now discussing, since the effects of vitamines were not studied in their investigations. Reference to some of their results will be made later.

In arranging an experiment to study whether tumor tissue in rats is dependent upon vitamine from outside sources for its growth, it would seem that the ideal condition to be fulfilled would be to give the young animal a vitamine-free diet which would keep it in good general condition, but upon which it was unable to grow at all. Inoculation of such an animal with virulent tumor tissue would then be made, and account taken of the number of "takes," and rate of growth as compared with controls upon a diet containing vitamine. Such a procedure is impossible with the present knowledge of diets. Upon a vitamine-free diet a young animal will rapidly decline in weight and will soon die. Even if this were not the case, the experiment would still be open to objection. The results obtained by Funk with pigeons render it highly probable that the tissues of all animals, so long as they live, contain very appreciable quantities of vitamines no matter how free the diet may have been from vitamine for a considerable period. Funk found that an extract of the tissues of pigeons fed upon polished rice until they died of beri-beri, would still readily cure other pigeons which had developed this disease upon similar diets.

Obviously, then, simply to take account of the "takes" and rate of growth of tumors inoculated into animals which are on a vitamine-free or a vitamine-restricted diet, will afford no answer whatever to the question which we are proposing to study. The tumor growth may be retarded simply because of the generally poor nutritive condition found within the host, or it may be retarded because of specific lack of vitamines. Or, conversely, the tumor might grow moderately well, due to its own synthesis of vitamine and greater avidity over the cells of the host for such circulating nutriment as is available. Or, it might be wholly unable to synthesize vitamine, but still might grow moderately well for a considerable period, owing to its greater avidity for both vitamines and food-stuffs available from the tissues and fluids of the animal. Under such conditions the host would probably die before very long, but it is quite conceivable that it could live for some weeks while the tumor was growing rapidly.

We believe that the objections above enumerated apply to practically all experiments involving studies of a deficient diet (whatever the specific deficiency of the diet may be) where the whole interpretation of the results of the experiment is made to depend upon counting the number of "takes" and measuring the size of the tumor at stated intervals. Such experiments may disclose a general effect of a diet upon the growth of a tumor, and at the same time give *no indication whatever* of the mechanism of the action. In the great majority of cases no definite effect may be noted upon the tumor growth simply because the tumor cells are able to make use of essentials for growth which are stored in the host's tissues and which may be wholly lacking in the animal's diet. It is quite possible that the slow progress in reaching a solution of the question of the relationship of nutritive factors to tumor growth has been partly due to the common practice of considering nothing but the relative size of the tumor as an element of importance.[1]

In planning our experiments, we have therefore attempted to avoid using the relative size or rate of growth of the tumors as the determining factor in interpreting our experiments. To do this we have assumed at the outset that a given quantity of normal tissue must have an approximately definite quantity of vitamine in order to attain a certain definite growth. In other words, x grams of tissue must use a definite amount (y grams) of vitamine to increase to $2x$ grams of tissue. If tumor tissue is also dependent upon vitamine from outside sources, then it too must fall in line with the above formula. We do not mean, nor need, to assume that the formula above given would hold more than approximately for different tissues, but we do believe that the general proposition that an increase in weight of normal tissue can be accomplished only in the presence of an increased amount of vitamines utilized is in accord with recent observations, and that it will hold also for tumor tissue, if this latter tissue is dependent upon vitamines from outside sources.

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[1] In their second paper, Sweet, Corson-White, and Saxon recorded curves giving the weights of their experimental animals plus tumors. The data offered cannot be interpreted from the point of view which we are advocating, nor do the authors attempt any such interpretation.

With this point of view in mind, the following experiment should throw light upon the question which we are trying to answer. Starting with a young and vigorous rat which is very far from having attained its complete growth (so that the tissues have a constant power to use a maximal quantity of vitamine), we must first determine, as exactly as may be possible, the amount of a particular material known to be very rich in vitamine which will, when fed daily, keep the animal alive in apparently good nutritive condition, but which will permit no appreciable increase in weight over a period of at least some weeks. Having thus determined the minimum quantity which will keep the animal in good condition, and at the same time permit no growth, the rat is inoculated with tumor tissue, and the results of this inoculation followed over a period which suffices to permit the tumor to reach a good size, providing it will grow at all. During this period the same food and the same quantity of vitamine are fed, and the weight of the animal is carefully recorded from day to day during the course of the growth of the tumor.

If the tumor does not grow at all, or grows very poorly in practically all such animals, we might assume at once that the tumor tissue was dependent upon vitamines for its growth, and, further, that it was unable, through increased "avidity" to draw vitamine from the tissues of its host—provided, of course, that the virulence of the tumor tissue was properly established. If, on the contrary, the tumor grows fairly well, or even very well, it is only necessary to note whether there is an increase in weight of the animal at least commensurate with that which we should expect from the tumor itself. If the animal plus the tumor increases in weight approximately in accordance with the weight of the tumor, it is obvious that this latter tissue is independent of an outside source of vitamine so far as its growth is concerned, since a quantity of vitamines is supplied in the food which will suffice only for the *maintenance* of the animal's original tissues. If, on the contrary, the tumor grows, but the *total weight of the animal plus the tumor remains unaltered*, then we should be justified in concluding that the tumor is dependent upon vitamine from outside sources, and that, through increased avidity, it is able to appropriate the vitamines of the host's tissues to its own uses, so that a given quantity of the host's tissue is practically transformed into tumor tissue. In this event there would be no appreciable change in weight in the animal, though the tumor might conceivably grow to a very considerable size before the death of the host. Another possibility would be that during the growth of the tumor, the weight of the animal would increase more rapidly than could be accounted for by the growth of the tumor. In this instance we should have to assume that the tumor produced a growth-promoting substance which escaped into the circulation and affected the growth of the host.

In order to meet the experimental conditions which have been outlined, it is necessary first to do some preliminary work to answer certain questions which arise at the outset of such experiments.

The diet which we adopted to constitute the vitamine-free ration in our experiments is one which has been studied in considerable detail by Funk and Macallum (5). This diet, as suggested by these authors, and employed by us, has the following composition: Casein[2] 22 per cent, cane sugar 10 per cent, starch 33 per cent, butter (ordinary) 30 per cent, agar 2 per cent, salt mixture (Osborne and Mendel (6)) 3 per cent. Funk and Macallum found that young rats fed on this diet lost in weight and died after about thirty to forty days. If 2 or 6 per cent of dried brewer's yeast was added to the ration, the rats grew normally for a period of at least two or three weeks. In their later paper these authors report experiments in which their animals grew normally for from forty to sixty days on the diet containing dried yeast (and in which lard partially replaced the butter), and in which good growth was maintained for about one hundred days when the diet was changed occasionally, substituting moist yeast for dry, or changing the percentage of yeast fed.

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[2] The casein is purified from the commercial product by repeated extraction with boiling 95 per cent alcohol.

In studying the suitability of the Funk-Macallum diet for our experiments, it was necessary to ascertain: (1) whether we could essentially duplicate their earlier results; (2) whether the diet plus yeast is adequate for growth over a considerable period,[3] and (3) whether yeast fed separately, i.e., not mixed with food, but given in a separate container, would be as effective as the yeast when mixed with the food. This latter point is of especial significance in connection with our work, in which we wish to control closely the vitamine intake. This obviously cannot be done where the food contains vitamines, since under such conditions the food intake controls the vitamine intake. The point is furthermore of importance in answering the more general question as to whether the stimulating effect of such a strongly smelling and tasting substance as yeast might be due to rendering the food more agreeable (through taste and odor) to the animals, so that they eat more, and hence grow more; or whether it has a specific effect within the organism.

An experiment to study these points over a considerable period of time has been carried out. Forty-two young rats, weighing approximately 25 grams each, were divided into one experimental group and one control group, of twenty-one rats each. The animals were kept in separate cages which were thoroughly cleaned at frequent intervals. The control rats were fed white bread and water ad libitum with the addition of cabbage or kale every second or third day. Such a diet is, we believe, quite commonly employed with animals kept in laboratories for experimental tumor work. The experimental animals received the Funk-Macallum diet above mentioned, with water, ad libitum, and in addition, varying quantities of dried brewer's yeast over different periods of the experiment. Where the quantity of yeast fed was below 1 gram per day, the yeast was given in the form of a thorough mixture with butter. By varying the percentage of yeast in the butter, it is possible to feed very small quantities of yeast per day with comparative accuracy and with ease of manipulation. Since the diet contained a high percentage (30 per cent) of butter, there is no reason to believe that the small additional quantity of butter fed with the yeast can have any special effect.

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[3] As mentioned above, this second question is really settled in the later paper of Funk and Macallum, which had not appeared when our work was started.

The experiment was continued over a period of one hundred and thirty-two days. Each animal was weighed individually and the weight recorded every four days. Early in the experiment a few of the animals on the special diet refused to eat the yeast, and died within the first week or two. No further deaths occurred among this group. In the controls there were no deaths during the experiment. At the end of the one hundred and thirty-two days, there remained alive twenty-one control animals, of which seven were females and fourteen males. Of the experimented animals seventeen remained alive at the end of the experiment, of which eight were females and nine were males.

The results of the experiment are summarized in Chart I. We have averaged together males of each group and females of each group. This is allowable only where (as in the present case) the results are very clear-cut, and where large variations among the members of each group do not occur. Thus out of the fourteen males in the control lot, as compared with nine males in the experimental lot, only one of the former exceeded in weight the lightest of the experimental animals. Among the females the lightest experimental animal weighed as much as the heaviest control animal.

An inspection of Chart I reveals several points of interest. The growth of the control animals is slow and regular throughout the entire period. At the end of the one hundred and thirty-two days the females of the control lot had practically ceased to grow, while the males were still noticeably increasing in weight. In the case of the experimental animals the rate of growth during the early periods appears to parallel the quantity of yeast fed, but only within certain limits. From the 1st to the 14th day each rat received 5 mgm. of yeast. There is a marked growth of the males during this period, and very little growth of the females. Probably this difference is accidental. From the 14th to the 28th day, each animal received 50 mgm. of yeast. The females show some improvement in growth during this period, while the males are almost unaffected. From the 28th to the 38th day, 200 mgm. of yeast were fed to each animal daily. The effect on the rate of growth is remarkable. During this ten-day period the animals almost doubled in weight, and the females caught up with and even surpassed the males. It is only during this period of ten days out of the entire experiment that the growth curves of the experimental animals

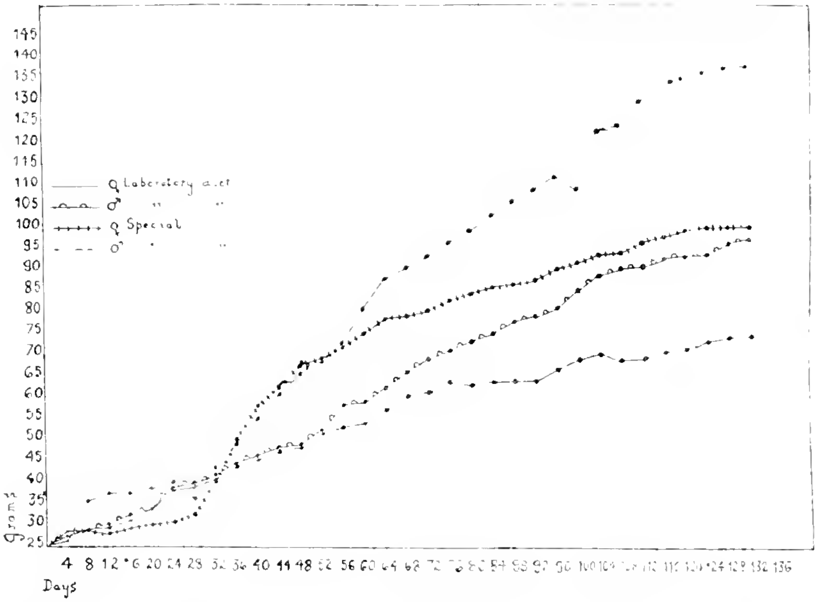

Chart I. Growth Curve of Two Lots of White Rats Over a Prolonged Period

Those on the laboratory diet received white bread and cabbage or kale. The special diet consisted of purified casein, cane sugar, butter and inorganic salts, with varying amounts of yeast fed separately. From the 1st to the 14th day these animals each received 5 mgm. of yeast daily, from the 14th to the 28th day 50 mgm. daily, from the 28th to the 38th day 200 mgm. daily, and for the remainder of the experiment 1 gram of yeast daily.

approximate closely that for normal rats as adopted by McCollum and others. From the 38th day to the end of the experiment, each animal received 1 gram of yeast daily. Many of the animals would not eat the whole of this yeast allotment, particularly toward the end of the experiment.

It will be noted that the increase in yeast from 200 mgm. to 1 gram per day is not accompanied by any increase in the rate of growth. On the contrary, the rapid growth of the preceding period is not long maintained, and falls off markedly for the females very shortly, and for the males a little later. Whether the optimum growth secured with 200 mgm. of yeast daily was only a chance result in this experiment can be definitely decided only by future work. But since the data represents about twenty animals, and since the effects appear to be so definite, we are inclined to accept the view that an "optimum" yeast intake can be found, and that this may be well below the maximum which the animal will eat.

As the result of their attempts to obtain growth over long periods with diets containing yeast, Funk and Macallum came to the conclusion that "it seems also possible that yeast on account of its high content in purines, and perhaps other constituents, is not an ideal addition in experiments of long duration, even in spite of its marked growth-promoting power." It would seem very possible, according to our results, that with careful regulation of the yeast intake the disadvantage of this substance as a growth-promoting agent over long periods of time might be minimized.

Certain other points in connection with the experiment recorded in Chart I are of interest. It is noteworthy that neither the experimental nor the control lot of animals attained nearly the weight to be expected in normal rats fed upon wholly adequate (normal) diets, as reported by McCollum, Mendel and Osborne, and Donaldson. At the termination of our experiment, the "expected" weight for our male animals would have been about 220 grams, of the females about 170 grams. This would seem to imply that both the diets employed are quite deficient as regards growth-promoting substances. Certainly the diet used for our controls (bread and cabbage) is low in protein, and we should be inclined to ascribe the slow and seemingly incomplete growth of the animals wholly to a protein deficiency, were it not that previous investigators have reported similar results upon diets against which this criticism could not be offered. Thus Sweet, Corson-White, and Saxon, in their second paper criticize the conclusions of Van Alstyne and Beebe (7) regarding the inhibiting effect of deprivation of carbohydrates upon the growth of experimental tumors in rats, upon the ground that neither the experimental nor the control animals employed by Van Alstyne and Beebe grew at all normally—hence these latter investigators were studying simply the relative effects of two diets, each of which was partially deficient for growth. It is true that the animals of Van Alstyne and Beebe did not grow normally, nor anything like normally, but that does not justify the conclusion of Sweet, Corson-White, and Saxon that the diets of these animals were deficient. That further work would be necessary to justify such an assumption is shown by the figures on growth reported by Sweet, Corson-White, and Saxon themselves for some of their own animals. These were presumably upon a diet of bread, oats, wheat, rice, corn, sunflower seed, and water, which must be regarded as complete in all ways. Yet these animals required a period of 21 weeks to increase from a weight of 50 grams to about 110 grams, which is approximately half the rate of growth "expected" for normal animals on an adequate diet. In a further period of six weeks, the animals declined in weight to about 90 grams, when, if normal and on an adequate diet, they would have increased to about 220 grams.[4] It is true that the rats of Sweet, Corson-White, and Saxon were abnormal in the respect that they carried tumors, but there is nothing recorded to show that rats developing tumors of considerable size thereby lose the power to grow at an approximately normal rate, even when the weight of the tumor is included. It would seem that some other factor is concerned in these results and in those which we illustrate in Chart I.

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[4] In recording the growth curve of their animals, Sweet, Corson-White, and Saxon make no reference to the sex of their animals.

The failure to grow at a normal rate exhibited by the rats of Sweet, Corson-White, and Saxon, and by our own animals, must rest rather upon a difference in the rats themselves than upon a deficiency in diet.

That this is probably the case is in accord with the observations of numerous workers who have carefully studied the growth problem in rats. Thus Funk and Macallum state that in their studies it was necessary to reject 80 per cent of the rats obtained from Toronto dealers because of physical defects which developed after initiation of the experiment; and Funk (8) states that animals furnished by dealers were unsuited to his work, and that the difficulties were overcome by employing rats bred in the laboratory *under proper dietary conditions.* (Italics ours.)

In view of such results, it is indeed questionable whether animals normal in their capacity for growth as determined by McCollum, Mendel and Osborne, and others, are to be obtained from dealers, or are to be found in many laboratories for the study of transmissible tumors. Apparently such animals must be bred under special conditions of diet. Conversely, it might be pertinent to ask whether the growth curves reported by the investigators for such animals are not maximal rather than normal,—since breeding under ideal nutritive conditions may improve the power of the offspring to grow.

Up to the present we have confined our experiments to animals purchased from standard dealers who supply many laboratories with animals for experimental cancer work. A little later we hope to study the effects of inoculating with tumor tissue animals having maximal growth rates.

Before leaving our first experiment, we wish to refer to the question of the general appearance and well-being of our animals. The control lot appeared to be in excellent condition throughout the experiment, with no detectable signs of inadequate nutrition. Infections were absent among this group, except in one animal which developed an eye infection after some weeks, and the fur of the animals was abundant, white, and soft.

In the case of the experimental animals, there was complete freedom from infectious disease, and the animals appeared to be in excellent nutritive condition in all respects save one. This point was the appearance and texture of the fur. The hair was abundant in all members of the group, but in every animal upon the special diet it was coarse and stiff, and slightly yellowish in color, as opposed to the soft and snow-white fur of the controls. This difference was so marked that with any rat of the entire two series a hasty inspection sufficed to indicate the diet on which it had been fed.

Thus the special diet, although far better suited for the growth of the rats than was the diet of the controls, was at the same time not so satisfactory in at least one respect.

A discussion of this point would lead us too far into a theoretical consideration of the vitamines. It will be sufficient for the present to point out that the difficulty with the diet is seemingly not connected with any toxicity associated with the yeast. We reach this tentative conclusion from the fact that the coarseness of the hair developed within a week of the beginning of the experiment, when the quantity of yeast fed was very small; also, from the further fact that we have seen it upon the same general diet when the quantity of yeast fed was less than 3 mgm. per day. An explanation of the difficulty with the diet which naturally occurs to one is that it may be associated with the probable absence of cystine. We hope to test this explanation in later experiments.

The two further experiments which we wish to report in the present paper were more definitely directed toward the question of vitamine relationship to tumor growth than the one we have been discussing. In these experiments we have employed the same diets as described for the preceding experiment, but in the case of our experimental animals we have attempted to reduce the yeast intake to the lowest level compatible with the general well-being of the animal, and one upon which it would not grow appreciably.

Chart II illustrates an experiment which is, we believe, the first on record designed to determine the minimal intake of a vitamine-rich constituent, essential to the growth and to the maintenance of young rats. The animals, twenty-four in number with an average weight of about 30 grams, were first upon a bread diet for a few days. They began to grow rapidly, and were then changed to the diet of casein, butter, and salt mixture described under the first experiment reported in this paper. The progress of the animals can be followed in Chart II, which represents an average of the twenty-four animals. With as large a group of small animals as this, a variation of a gram or two in weight for the average is of distinct significance. There was no separate charting of males and females because the rate of growth was not to be especially considered in these experiments.

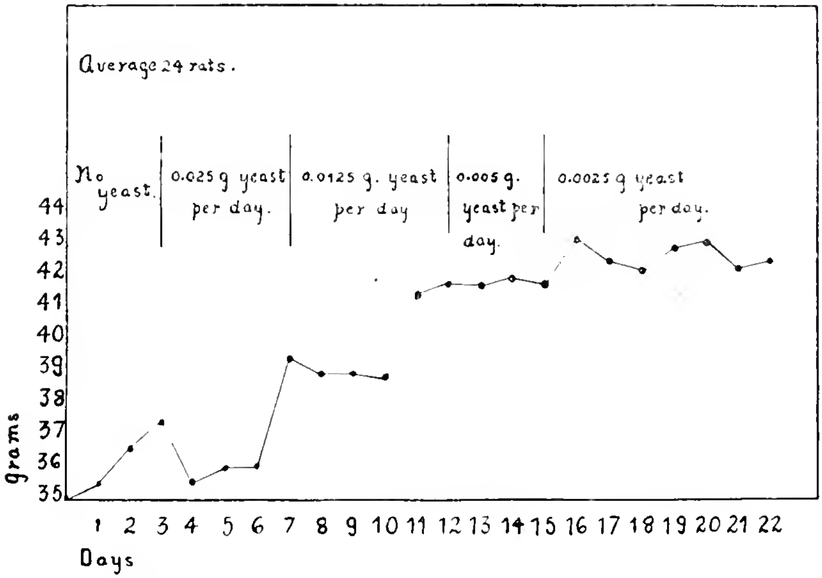

CHART II. AN EXPERIMENT TO DETERMINE THE MINIMAL VITAMINE INTAKE COMPATIBLE WITH PROPER MAINTENANCE, BUT UPON WHICH THERE CAN BE NO GROWTH

The animals (white rats) were fed upon a diet of purified casein, cane sugar, butter and inorganic salts, and in addition each animal received the quantity of yeast indicated.

It will be noted from the chart that the first three days the rats continue to grow without any yeast in the diet—apparently an after-effect of the bread diet. From the third to the fourth day, however, there is a sharp drop in weight which is promptly checked, and soon wholly recovered from when 25 mgm. of yeast (in mixture with butter) was fed once a day, separately from the rest of the food. In spite of the decreasing amounts of yeast fed, there is a definite tendency to grow until a minimum of 2.5 mgm. of yeast is reached, when the weight is constant, and the animals remain in good condition for at least a week.[5]

The animals were then planted (by trocar) with the Buffalo sarcoma, and were divided into two lots of twelve animals each. Lot I (the experimental lot) was continued on the same diet as was previously given, while Lot II (controls) was placed upon a diet of bread and cabbage.

Difficulties were encountered in the handling of the experimental lot as regards the quantity of yeast necessary to keep the animals alive. Apparently there is a definite drain upon the animals as the tumor cells begin to grow which is especially notable for the lighter weight animals. In spite of an increased allowance of yeast on certain days, four out of the twelve animals died within the first three weeks after planting. The average weight of the remaining eight animals at the time of planting was 48 grams. At the end of three weeks all eight animals had tumors ranging in size from a trace to 1600 cmm. in content[6] with an average size of 850 cmm. The average weight of the animals (plus the tumors) at the end of these three weeks was 47 grams (as compared to an average weight at the time of planting of 48 grams).

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[5] Funk and Macallum report experiments in which their animals remained alive, and at almost constant weight for two or three weeks on this diet with no yeast at all. Apparently their animals were far stronger and of greater vitality than ours, as we have been unable to obtain similar results in a number of attempts which we have made. From a reference to our discussion earlier in this paper, it will be seen that Funk and Macallum certainly worked with quite a different class of rats from those which we have employed. In duplicating this experiment, we have found that the quantity of yeast necessary for maintenance varies both with the animals employed and with the sample of yeast used. The chart represents minimal figures so far as our results go up to the present time. In some experiments the animals almost wholly refuse to eat the yeast, while in others (as in the one illustrated in Chart II) all the animals eat the yeast quantitatively, practically as soon as it is placed in the cage. The yeast is always prepared in the same way (by pressing, drying, and grinding fresh brewer's yeast) but seemingly different samples have different growth-promoting powers.

The control animals, fed upon bread and cabbage after being planted, increased rapidly in weight, and grew tumors of very fair size within the three-week period. Nine of the twelve animals planted remained alive at the end of three weeks after inoculation: these were eight "takes," and two regressions, leaving six tumors. These varied in size from 480 cmm. content to about 11,500 cmm., with an average size of about 6000 cmm. The weight of these animals (plus tumors) increased from an average of 44 grams at the time of planting to an average of 77 grams three weeks after inoculation and the initiation of the bread and cabbage diet. This is fully up to the normal rate of growth for white rats over this three-week period.

It is obvious that this experiment would warrant no conclusion concerning the necessity of vitamine for tumor growth. It is true that the difference in size of the tumors in the two lots is striking. The tumors of the controls were between seven and eight times as large as those of the experimental animal, as will be seen from the following table.

NUMBER OF ANIMAL	SIZE OF TUMOR AT END OF THREE WEEKS	
	Special diet	Laboratory diet
	cmm.	*cmm.*
1	720	10,000
2	160	4,400
3	660	7,200
4	1,638	0
5	800	3,800
6	Trace	0
7	980	0
8	840	11,500
9		480

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[6] Computed by measuring growth in three directions and multiplying.

It will be noted that except for one tumor (which was regressing) the smallest growth among the control animals was more than twice as large as the largest one among the controls.

We do not, however, regard these results as of any special significance, since they might be due to any of several factors mentioned earlier in this paper besides the one we are trying to investigate. They tend, however to indicate that vitamine from outside sources may be of importance for tumor growth. The crucial test of whether there was an increase in weight of the tumor bearing animals without appreciable increase in the vitamine ingested, could not be satisfactorily applied in this experiment, since the tumor growth in the experimental animals was so restricted that it would not, by itself, have caused any appreciable increase in the total weight of the animal.

The experiment is, however, of some interest in connection with the following experiment which is essentially a duplicate of it, but in which diametrically opposite results are obtained as regards the rate of growth of the tumors on the two diets.

The diets and general conditions of this experiment were the same as for the preceding, except that the preliminary period on the special diet covered only six days before the animals were inoculated. There was a higher mortality among the experimental animals, possibly due to the higher virulence of the tumor tissue among these animals, as compared with those in the preceding experiment. Starting with seventeen rats on the special diet, and with a minimal yeast intake, there remained alive eight animals four weeks after inoculation. Eleven out of fifteen "control" animals remained alive four weeks after inoculation.

Of the eight experimental animals, seven had tumors at the end of the four week period. The tumors ranged in size from 600 to 28,000 cmm., with an average size of about 8000 cmm. The average weight of the animals remained constant (within two or three grams) throughout the entire period of the experiment.

Of the eleven control animals, seven had tumors at the end of the experiment, which ranged in size from a trace to about 6000 cmm., with an average size of about 2200 cmm. The average weight of this series increased from 27 grams at the time of planting to 54 grams during the experimental period.

In this experiment the animals on the vitamine-restricted diet developed tumors of very considerable size within a period of about four weeks. The fact that these tumors were about four times as large as those developed by the controls, would, according to the usual method of interpretation, be taken to indicate that the tumor can grow independently of outside source of vitamine, thus being directly contradictory to the preceding experiment. But when we apply the test proposed at the outset of these experiments it is plain that no such conclusion is justified. In fact the experiment is wholly independent of the controls. If the tumor could synthesize its own vitamine, then the total weight of the animals should have shown an increase in some proportion to the growth of the tumor. In no single case did this occur. The rat developing the largest tumor was the heaviest animal in either lot, and weighed 52 grams at the time of inoculation. This animal showed, it is true, the maximal gain of any of the experimental animals, and gained 6 grams in the first ten days after inoculation, having then a weight of 58 grams, and a small tumor (under 2000 cmm.). At the end of the experiment the animal's weight (plus the tumor) was still 58 grams, but the tumor had increased to a weight of nearly 20 grams, or about one-third of the weight of animal plus tumor. The weights were even more constant for the other animals which developed tumors of very appreciable size, though not so large as this one.

The experiment serves to bring out, perhaps more clearly than has been done before, the remarkable avidity which tumor cells may show, since a very rapid growth of tumor has been obtained under conditions which *completely* prevented growth of the body tissue. It also shows that even a very rapidly growing experimental sarcoma does not liberate into the blood-stream any substance which may act as a stimulant to body growth in the absence of the necessary amount of chemical stimulant contained in yeast. We may tentatively assume that the tumor itself is also dependent upon this stimulant from outside sources, either from the food, or from the tissues of the host. We recognize fully the desirability of confirming these results by further experiments, and are continuing the work along this and similar lines. It should be noted however that our experiment as reported above, being independent of the rate of growth of the tumor, and therefore of controls, can afford positive evidence much more readily than those based upon the usual procedure of measuring rate of growth in experimental animal and control.

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THE ALKALINITY OF THE BLOOD IN MALIGNANCY AND OTHER PATHOLOGICAL CONDITIONS; TOGETHER WITH OBSERVATIONS ON THE RELATION OF THE ALKALINITY OF THE BLOOD TO BAROMETRIC PRESSURE

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The following pages contain the results of a study of the hydrogen ion concentration of blood serum in malignant disease and various other pathological conditions. The general conclusion of this study has been the discovery that while the alkalinity of the blood serum may be increased or diminished in various diseases, yet almost without exception the cases of malignant disease, that is, cases of cancer of the skin and internal organs, and of sarcoma, show a characteristic change in the reaction of the blood serum. This change consists in a marked increase in the alkalinity of the serum. In contrast to these findings are the results obtained in cases of tuberculosis, rheumatism, pemphigus, nephritis, and heart lesions accompanied by dyspnoea, in which the acidity of the serum is increased. The increase in alkalinity of the serum in malignancy is so constant and of so marked a character when the growth occurs in the internal organs that it is believed that it may furnish an additional diagnostic aid of value, particularly in early stages in internal organs; and the possible significance of the increased alkalinity in the production of the disease itself or its rapid progress naturally suggests itself.

In the course of the investigation the attempt was made to measure the hydrogen ion concentration, that is the alkalinity, of the whole blood, an attempt which had been made by various observers. When the whole blood was used it was found that there were wide variations from time to time in the same individual, variations which would conceal the characteristic changes accompanying disease if any such changes existed. An investigation of the causes of these variations resulted in the discovery that the alkalinity of the whole blood was strongly affected by barometric pressure, in the way which will be described further on. The exact mechanism of this relation between alkalinity and pressure is under investigation. The difficulty it introduced was avoided by using blood serum rather than the whole blood, the blood serum not varying in reaction with the atmospheric pressure. It is believed that it is this variation of the alkalinity of the whole blood with pressure and possibly other external conditions which has masked the characteristic change in alkalinity accompanying malignancy from previous investigators who have nearly all examined only the whole blood.

The alkalinity of the blood and other fluids of the body has been so long recognized as being of fundamental importance in the functioning of all the tissues of the body that many investigations have been made of its variation in disease with the expectation of finding that it underwent some characteristic deviations in different diseases. These investigations were made in the first instance by means of titration of the blood with acid, and certain important facts were discovered by this method. The method itself, however, is a very coarse one, and is incapable of showing those changes in the actual alkalinity of the blood which are expressed by or due to the hydroxyl ions it contains. With the introduction of the physico-chemical method of measuring the actual alkalinity, as distinct from the titrateable alkalinity, these attempts were renewed; but they have not so far led to any definite conclusions for the reasons which will be shown presently. Before going into a discussion of the results which others have obtained, it will be interesting very briefly to consider some of the indications of the great importance of the reaction of the blood for all physiological processes, and accordingly for all pathological processes as well. It has been found, for example, that very minute changes in the alkalinity of blood are responsible for the changes in activity of the respiratory center of the body (1); an increase in alkalinity depresses that center, a fall in alkalinity stimulates it to greater exertions to remove from the blood the carbon dioxide, one of the substances which is responsible for the diminution of alkalinity. Not only is the respiratory center intimately dependent for its activity on the number of hydrogen or hydroxyl ions in the blood, but so also is the activity of many other, if not all centers of the nervous system. But in many cases, at any rate, the rule appears to be that an increase in alkalinity serves to stimulate those centers, rather than to depress them. The respiratory center is in this particular different in its behavior from many other parts of the nervous system. But not only is the nervous system thus dependent on very small changes in alkalintiy; so also are all the other tissues and organs of the body. Acidity depresses or retards recovery from fatigue, whereas alkalinity hastens recovery. Rigor mortis, for example, may be prevented by preventing acidity. And numerous other examples of the importance of the alkalinity for muscle might be given. One of the most interesting of the examples of the importance of alkalinity for proper functioning is that given us by the behavior of haemoglobin (2). The affinity of this substance for oxygen is increased by a very small increase in alkalinity, and correspondingly diminished by a very slight increase in acidity. Thus the respiration of the whole body is dependent upon very small changes in the hydrogen ion content of the blood, changes so small that one might easily overlook them, but changes of profound importance to the organism. Another proof of the importance in metabolism of this alkalinity is given by the activity of the various ferments of the blood and tissues. Most of these show a remarkable sensitiveness to such variations. Thus the power of the ferment invertin (3) is altered many hundred per cent by a trifling variation in the acidity or alkalinity of the liquid in which it is, variations so small that they lie well within the normal variations occurring in cells and liquids of the body. But one of the most instructive and remarkable of all the evidences we have for the importance of this property is the dependence of growth, or cell division, on alkalinity, for this peculiarity touches so closely what we have found in malignant disease. It has been found, for example, in studying the rate of cell division in eggs, and the rate of their respiration, that both of these fundamental properties are markedly increased by a small increase in the alkalinity of the sea or other water in which the egg is, and that they are correspondingly diminished by an increase in the acidity (4). These few examples will make it clear that in dealing with the alkalinity, that is, with the hydroxyl ion content of the blood, we are dealing with one of the most important factors in that fluid which conditions growth and all other functions. In measuring the hydroxyl ions we are measuring one of the most important of the properties of the blood. It would indeed be astonishing if there were no characteristic changes in this property of the blood in health and disease.

There have been several investigations of the hydroxyl ion content of the blood in health and disease. The general result of this study has been a failure to detect any characteristic changes in different diseases. It is true that increases or decreases have been reported at times, but these changes have not been uniform for any single disease, and they are as a rule not greater than the variations which are found in the blood of a single individual at different times. The cause of these variations has not yet been completely cleared up. Among the investigators who have made extensive studies of the hydrogen ion content of the blood in disease, special mention may be made of Michaelis (5), who has done so much for the advancement of our knowledge in this field. The literature has been summarized in the book recently published by this investigator, so that we shall not go into it at any length here. But we may say that Michaelis, as well as his predecessors and those who have worked more recently on the problem, have not been able to show that the alkalinity was altered always in the same direction in any disease studied. They have not found any correlation between alkalinity and malignancy. We also in our early investigations ran across these same wide variations, which destroyed any semblance of uniformity in our results, and it was only after we had succeeded in discovering their origin and eliminating them by our experimental procedure that we observed the remarkable uniformity recorded in this paper. The results of some investigators, such as Rolly (6) and Salge (7), show such wide variations that Michaelis' suggestion that inaccuracies in the method may be responsible for some of them, seems not unjustifiable. Most observers have determined the alkalinity of the whole blood, in which both corpuscles and plasma play a part. Among the more recent publications may be mentioned Peabody (8), who found a marked increase in acidity in advanced cases of chronic nephritis. Other investigators, however, have used the serum as in the observations here. Thus Benedict (9), using the gas-chain method of determining alkalinity, in a number of cases of diabetes was able to discover only slight changes from the normal serum reaction, a result which is at variance at first glance, but not really, with what we have found. His cases differed from ours in that most of his patients showed acidosis. In diabetic and uraemic coma studied by the same method Kreibich (10) also reported a decrease in the alkalinity of the serum. Moore and Wilson (11) worked on the alkalinity of the serum of the blood in cases of cancer, and they found a small but very constant increase in the alkalinity in this disease as well as in diabetes, although they determined the alkalinity not by the method we have used, but by titration. Our results are, however, in accord with theirs.

METHOD

The method employed in these experiments was the following: The blood to be tested was obtained from one of the veins in the cubital space at the elbow, and was drawn by a needle inserted in the vein directly into a Michaelis hydrogen electrode containing hirudin in normal saline to prevent clotting, and another sample was drawn into a test tube in which it was immediately defibrinated. The defibrinated blood was centrifuged, and the serum was used for examination. All estimations were made electrically by the gas-chain method, with apparatus and according to the method recently described in detail by Michaelis (5).

For the measurements of the hydrogen ion content of the serum, both the Michaelis and the Walpole electrodes were used; but for blood, only the former. A sketch of both forms of the electrodes is given in figures 1 and 2, and reference to these will elucidate the description of the procedure employed, and especially that in obtaining serum with a minimal amount of carbon dioxide.

In the Walpole electrode (fig. 1) in which a T-tube is blown into the electrode tube, one branch of the T connects with the hydrogen generator and is fitted with a stop-cock *B*, while to the other is attached, by means of a rubber tube, a glass Luer syringe. To use this electrode the piston of the syringe is put completely into the barrel and the stop-cock, *B*, is opened so that a continuous stream of hydrogen passes through the solution into which the point, *A*, of the electrode dips. When hydrogen is passed into the solution long enough to have driven off the carbon dioxide so that only a minimal amount remains,

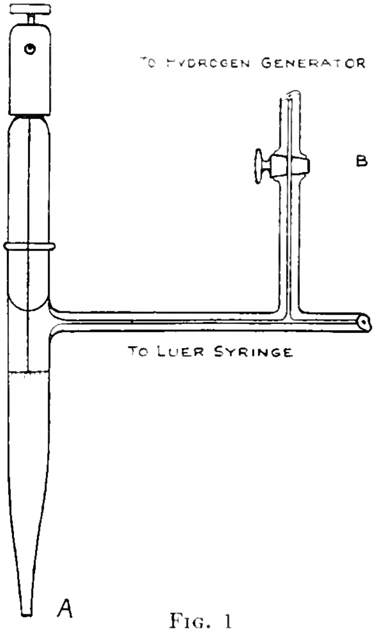

FIG. 1

the stop-cock, *B*, is closed and, by drawing out the piston of the syringe the serum is sucked up into the electrode until only a small amount of the platinum wire dips into the liquid. The whole electrode is then placed so that its point, *A*, is slightly immersed in the potassium chloride solution used in connection with the whole apparatus. The arrangement of the remainder of the apparatus for measuring the hydrogen ions and of which the potassium chloride solution forms a part, is that described by Michaelis, and will not be repeated here. The presence of air bubbles in the lower end of the tube is guarded against by pressure on the syringe piston, which also drives out small amounts of serum until only the extreme point of the electrode wire touches the solution. In order to insure constant readings and be certain that the carbon dioxide has been reduced to a minimum, this whole procedure was repeated at least four times and in many cases checked by measurements made on the serum with the Michaelis electrode tube.

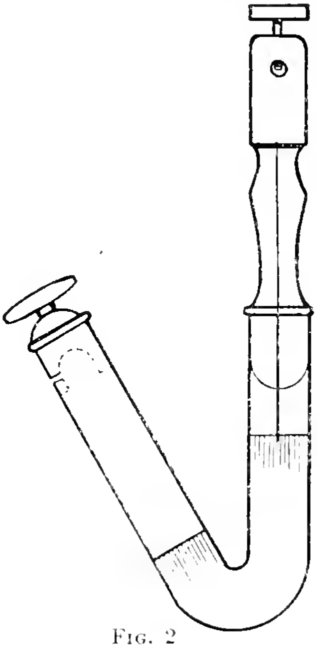

FIG. 2

With this apparatus (fig. 2) the tube was filled about three-quarters with serum and hydrogen then bubbled in until only the tip of the platinum wire was immersed in the liquid. The tube was then completely filled and closed with a stopper, which, by means of the opening it contains, permits this to be done without the introduction of any air. Equilibrium of the gas in the electrode was obtained by moving the electrode tube so that the bubble of gas about the platinum wire passed back and forth through the liquid about two hundred times. This bubble of hydrogen was then passed out of the tube and the procedure just described was repeated four or five times with new bubbles of hydrogen. By this means the carbon dioxide content of the serum was reduced to a minimum in the Michaelis electrode also.

The method used for whole blood was similar to that used for serum with the Michaelis electrode, except that a solution of 0.85 per cent sodium chloride containing hirudin was put in the tube so that it partially filled it, hydrogen bubbled in, and about an equal quantity of blood drawn from the vein at the elbow was then introduced so that it filled the electrode. The electrodes used throughout were of the same size, and in all experiments as nearly as possible equal amounts of sodium chloride solution were used. Readings on both blood and sera were made as soon as possible after the blood was removed from the vein. This time varied between thirty minutes and four or five hours, depending upon whether the blood came from one hospital or another. Corrections were made for the temperature at which the measurements were made, according to the formula given by Sörensen (12). It ought to be said that controls showed that this difference in interval after drawing introduced no error into the results.

The dilution of the blood by means of sodium chloride solution containing hirudin will naturally arouse suspicion that this might introduce some alteration in the hydrogen ion content of the blood. Michaelis and Rona (13) state, however, after an investigation of this possibility, that this is not the case. At any rate as the procedure followed was always the same in the examination of the whole blood, it is to be presumed that if any changes were introduced they would affect all samples equally and would not interfere with comparative studies of the blood in different conditions in such studies as were made here. Of course in the more important part of this work, in which only serum undiluted was used, this possible source of error was entirely avoided. A serious difficulty in dealing with whole blood and its hydrogen ion content arises from the possible variation in its carbon dioxide content as was first pointed out by Höber (14). This affects the alkalinity of whole blood very strikingly, but this fact does not affect the conclusions which have been drawn in this paper.

I. THE CORRELATION BETWEEN THE ALKALINITY OF THE WHOLE BLOOD AND THE BAROMETRIC PRESSURE

At the start of the investigation I attempted to study the variation of the hydrogen ion content of the whole blood in various diseases, and in particular, in malignancy. In the course of these studies it was found that when samples of blood were taken on the same day from a number of patients suffering from the same or different diseases, these samples were found to contain about the same number of hydrogen ions. In some cases the determinations showed exactly the same values in different patients. Similar tests made on another day would again show the unexpected result, that, irrespective of the nature of the disease, the various patients would show the same hydrogen ion content in the blood; but this number might be either higher, lower, or the same as that shown by all the samples examined on other days. That is, the alkalinity of a series taken on the same day was constant, but varied from day to day. This wholly unexpected result was at first extremely discouraging, for it seemed to indicate that there were no variations of alkalinity of the blood with disease in such a manner, at any rate, as to be characteristic of any disease. And this was the result that most, if not all previous observers had reached. However, my curiosity was aroused by this peculiar variation from day to day of the group examined and I cast about for some explanation of it. These variations might be due to physical conditions, such as temperature, which affected the patients equally, or they might be due to conditions which varied from day to day and which influenced the readings of the electrodes. The first hypothesis was that it was something affecting the electrodes rather than the patients. It could not be temperature, because the temperature of the room remained nearly constant, and in the second place what change there was, had already been corrected for. It might be that the barometric pressure influenced the pressure of the hydrogen gas in the electrode and so affected the readings. However, the variation due to this cause is comparatively small, and has been recently estimated by McClendon (15) to be about 1.7 millivolts for a difference of pressure from 760 to 740, while the actual value found is 10 or 11 millivolts. Further, that this was not the explanation was shown by the fact that the serum itself showed no similar variations from day to day. It was evidently something which acted on the patients altering the alkalinity of their blood. Temperature being excluded by the rather uniform temperature of the hospital wards, diet occurred as a possibility, for all the patients were on the same diet. An examination of this possibility showed, however, that there was no discernable relation between diet and alkalinity. It might also have happened that the blood was more alkaline just after a meal than at other times, and that drawing blood at different times during the day might account for the variations obtained; but this possibility was also investigated, and found not to be the explanation. Practically all the samples were drawn between nine and ten o'clock in the morning.

It also occurred to me that it might be a psychical effect since I had already found in work with Dr. Crile (16) that fright and anger in cats, and fright in rabbits, affected the alkalinity of the whole blood. But there were no psychical phenomena of the kind affecting all patients on the same day which could be discovered.

It finally occurred to me that there might be a correlation with the barometric pressure, and on investigating this possibility I found the remarkable relationship exhibited in the figures of the last three columns in the tables which follow, and in the chart shown in figure 3. I found that with a low baro-

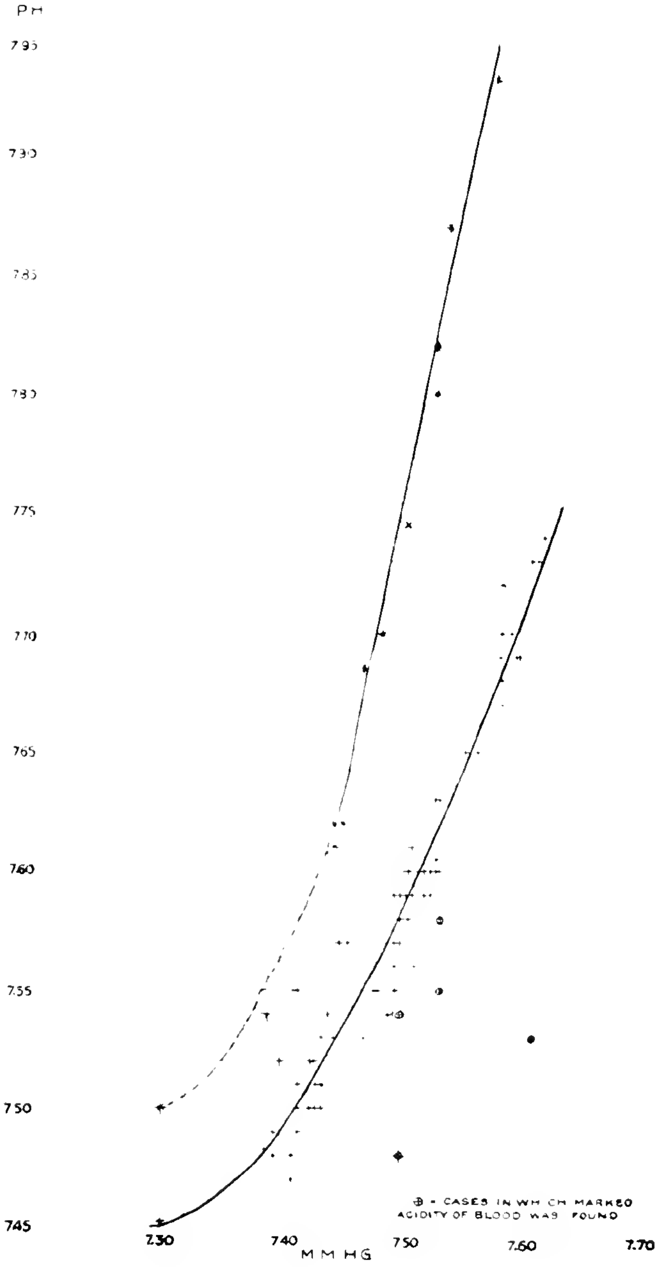
PH
7.95
7.90
7.85
7.80
7.75
7.70
7.65
7.60
7.55
7.50
7.45
7.30
7.40
7.50
7.60
7.70
MM HG
⊕ = CASES IN WHICH MARKED ACIDITY OF BLOOD WAS FOUND

FIG. 3

metric pressure, namely, a pressure of about 730 mm. of mercury, the concentration of hydrogen ions was 3.6×10^{-8}; that it fell as the pressure rose, so that at the highest barometric pressure observed, namely, 762, it was 1.84×10^{-8}. These two figures represent the extreme values found by Michaelis (5) in his measurements of blood from patients suffering with various diseases. In the lower curve of figure 3, the abscissa has plotted on it the barometric pressure in millimeters, while the ordinate represents the value of pH, that is the common logarithm of the concentration of hydrogen ions expressed as positive, instead of a negative number. It will be noticed that while there are some variations in the different cases of which pH is given, that is, not all bloods have exactly the same alkalinity at the same barometric pressure, yet these variations (except in a few cases to be mentioned later) are small, compared with those which are due to barometric pressure. That there is a general trend or advance of the values of alkalinity with the pressure is undoubted. And it would probably be possible to formulate the results in such a manner as to allow for this variation and reduce the alkalinity, just as the gas pressure is usually reduced to a standard barometric pressure, let us say, of 760 mm. of mercury. An attempt will be made to do this later.

The increase in the alkalinity of the blood with barometric pressure was a wholly unexpected observation, and the explanation of it cannot yet be given. The natural expectation would seem to be that with a rise in pressure there would be a rise in the carbon dioxide content of the blood with a resultant fall of alkalinity. It was first shown by Höber (14), and has since been confirmed by many other observers, that an increase in the carbon dioxide content produces a decrease in alkalinity. And it is also known that a reduction of barometric pressure generally reduces the carbon dioxide content of the alveolar air, and hence of the blood. So far as the carbon dioxide is concerned, therefore, it would seem that a rise in pressure should reduce alkalinity rather than increase it. It may be that the increase in the oxygen content of the blood brought about by the increase in pressure is sufficient to increase in some other way or other the alkalinity, and that this is more than sufficient to neutralize the effect of the carbon dioxide. Experimental evidence for this is not lacking. Milroy (17) found in cats, on increasing the oxygen ventilation of the lung, that there occurred a corresponding rise in the alkalinity of the blood; while similarly Barcroft and Orbeli (18) reported increased acidity as indicated by the haemoglobin dissociation curve, when they reduced the percentage of oxygen in the air breathed by the animal. Ryffel (19) also showed under decreased atmospheric pressure, increased lactic acid in the blood with a corresponding diminution in its hydroxyl ion content. It may be, however, that the alkalinity is due to some other reaction of the body of an unsuspected kind to variations of barometric pressure, so that while the increase in pressure would of itself tend to increase acidity, by some response of the body this change is met and overcompensated through the activity of some organs of the body or by the blood itself. In any case it is clear that this curious reaction of the alkalinity of the blood to barometric pressure opens up many possibilities which are worthy of careful study. Since the relation to barometric pressure appears only in the alkalinity of the whole blood and not in that of the serum, it is plain that the change, whatever its nature, must affect the corpuscles and not the serum. This would seem to correlate it either with the haemoglobin or the phospholipins in the corpuscles. It is apparently the acidity of the corpuscles which is altered, and not that of the serum. This is entirely analogous with observations which I have made on anaesthesia, and which will be reported in detail in a subsequent paper. In anaesthesia there is an increase in acidity of the whole blood, but there is no appreciable change in the serum. Again it appears to be the corpuscles which change their acidity.

As regards the shape of the curve it is clear that as it is not a straight line but more like the curve of an autocatalysis, a given increment of pressure does not affect the alkalinity to the same degree throughout the curve. When the pressure is between 740 and 760, a small increment in pressure produces the most marked influence, and as the pressure decreases below 740 the increment produces less and less effect. While there is some indication of a flattening of the upper end of the curve beyond 760, the opportunity did not occur of obtaining values of alkalinity with a pressure beyond 762.

The upper curve in figure 3 was obtained by replacing the hydrogen in the Michaelis electrode after it had come to equilibrium with a new bubble of hydrogen, allowing it to come to equilibrium and remeasuring. This was done for a certain number of cases which had given at the first reading results plotted in the lower curve. The effect of this procedure is to remove some of the carbon dioxide from the blood. The carbon dioxide passes into the hydrogen bubble and is removed when this is taken out and replaced by fresh hydrogen. The effects of thus reducing the carbon dioxide is shown in the marked increase in alkalinity, that is, in the increase in pH. But it will be noticed that these second readings do not change the character of the curve; they only change the position on the diagram.

If in introducing the hydrogen the second time more than a minimum amount of oxygen gets mixed with the blood in freeing the hydrogen tube of air, then the alkalinity is raised to a maximum amount for all the samples of blood. That is, the alkalinity goes as a maximum to about pH 7.95 or 8.00, depending on the amount of air introduced. This again looks as if the change in alkalinity associated with barometric pressure has some relation to the oxygen saturation of the blood rather than to the carbon dioxide content. But it will be clear that removal of carbon dioxide and increase of oxygen both act in the same direction, namely, to increase alkalinity.

II. THE ALKALINITY OF BLOOD SERUM IN MALIGNANT DISEASE AND OTHER PATHOLOGICAL CONDITIONS

The foregoing observations having led to the result that the blood as a whole showed no characteristic variations in alkalinity in disease, but that it underwent surprising fluctuations under various external conditions, led me to examine the alkalinity of the blood serum in the hope of finding there simpler, less complicated conditions. This hope was fulfilled, as will be shown.

Alkalinity of normal blood serum. Twelve cases of normal blood serum were examined. These are contained in table 1. It will be seen by an inspection of that table that the value of p_H was very constant, quite regardless of the barometric pressure, and it ranged from 7.89 to 8.02. Nearly all of them were very close to 7.93. A word may be said of the two extremes. The person G. P. who had the lowest value of pH had a compensated heart lesion. In other particulars she was normal. The highest value of 8.02 was obtained from a nurse who had an excessive amount of indican in the urine. After some weeks on a low protein diet and treatment with calomel and thymol, the urine became indican-free. The second reading made on the serum at this time had a normal value of pH 7.94. All of the cases examined were adults.

TABLE 1

NAME	SEX	SERUM		BLOOD		BAROMETRIC PRESSURE
		10^{-8}	pH	10^{-8}	pH	
						mm. Hg
G. P., normal	F.	1.30	7.89	2.26	7.65	756.5
M. L. M., normal	F.	1.25	7.91	3.60	7.45	730.0
M. C., normal	F.	1.22	7.92	1.88	7.73	761.5
H. J., normal	F.	1.22	7.92			
E. L. F., normal	F.	1.19	7.93			
F. C., normal	F.	1.19	7.93			
F. K., normal	M.	1.19	7.93			
F. S., normal	M.	1.16	7.94			
G. Q., normal	F.	1.16	7.94			
E. C. W., normal	F.	1.16	7.94			
L. M. L., normal	F.	1.16	7.94			
E. L. L., normal (indicanuria)	F.	0.97	8.02	1.93	7.72	758.5
E. L. L.		1.16	7.94	1.88	7.73	761.5

Alkalinity of blood serum in pregnancy. I examined the blood serum in eleven cases of pregnancy which were nearly full term. The results are put down in table 2. There was only a single one of these which varied more than 0.2 per cent from the usual value of 7.93. This one had a reading of 7.98. No opportunity was had of repeating this or of studying the case to discover whether there was any pathological lesion. It is evident that the development of the fetus does not alter the alkalinity of the blood serum of the mother. This is a point of considerable interest since we have in pregnancy the same rapid cell proliferation which occurs in cancer, and it might be supposed that this necessarily brought about the rise in alkalinity. It is also of value in diagnosis that the alkalinity does not increase in pregnancy, since a differentiation from a tumor is thus easier. Michaelis (5) reports that in the whole blood there is a slight increase in alkalinity on the average, although a few cases showed no more alkalinity than the normal. This difference found, however, was not sufficiently marked to be of value in the diagnosis of pregnancy. Since he was not aware of the influence of atmospheric pressure on the alkalinity of the blood, it is quite possible that the slight effects he reports may have been due to this and not to the pregnancy. Certainly our cases do not have any greater alkalinity than normal in the serum. The great constancy of the alkalinity in normal serum, so far as our results have gone, makes any deviation from that value the more significant.

TABLE 2*

NAME	AGE	CLINICAL DIAGNOSIS	SERUM		BLOOD		BAROMETRIC PRESSURE
			10^{-8}	pH	10^{-8}	pH	
							mm. Hg
N. W.	21	Normal pregnancy full term	1.24	7.91	2.99	7.53	744.5
G. P.	20	Normal pregnancy full term	1.24	7.91			752.5
E. W.	14	Normal pregnancy full term	1.22	7.92	2.99	7.53	744.5
C. H.	25	Normal pregnancy full term	1.22	7.92	2.99	7.53	744.5
M. B.	18	Normal pregnancy full term	1.22	7.92	2.99	7.53	744.5
A. D.	24	Normal pregnancy full term	1.22	7.92	2.99	7.53	744.5
A. D.	25	Normal pregnancy full term	1.19	7.93	3.06	7.52	744.5
A. D.	23	Normal pregnancy full term	1.19	7.93	2.55	7.60	752.5
K. H.	26	Normal pregnancy full term	1.19	7.93	2.60	7.59	752.5
A. F.	19	Normal pregnancy full term	1.16	7.94			752.5
B. P.	21	Normal pregnancy full term	1.06	7.98	2.55	7.60	752.5

* I am indebted to Dr. G. Gellhorn and Dr. F. J. Taussig for the above cases, which were obtained from their obstetrical services at the City Hospital.

Alkalinity of blood serum in syphilis. Fifteen cases of syphilis were examined and the results are embodied in table 3. The value of the alkalinity of the blood was found to be the same as in normal individuals, except in the last two, where complications occurred. These cases all gave strongly positive Wassermann reactions at the time of the examination with one exception, and this patient had a history of syphilis. This coincidence of the very positive Wassermann reaction without any change in the alkalinity of the blood, indicates that the changes responsible for the positive Wassermann do not depend upon a change in serum alkalinity.

TABLE 3

NAME	SEX	AGE	CLINICAL DIAGNOSIS		SERUM		BLOOD		BAROMETRIC PRESSURE
				Wassermann	10^{-8}	pH	10^{-8}	pH	
									mm. Hg
W. F. H......	M.	50	Syphilis	++++	1.29	7.89	2.55	7.60	753.3
L. L..........	M.	44	Syphilitic ulcer of leg	++++	1.29	7.89	2.32	7.74	762.0
J. A..........	M.	23	Syphilis	++++	1.27	7.90	2.92	7.54	749.6
W. W..........	M.	30	Syphilis	++++	1.27	7.90			
M. R..........	F.	21	Syphilis	++++	1.27	7.90	3.35	7.48	755.0
D. K..........	M.	40	Syphilis	++++	1.22	7.92	2.79	7.56	749.6
D. A..........	M.	33	Syphilis	++++	1.22	7.92	3.13	7.51	743.5
J. V..........	M.	25	Syphilis	++++	1.16	7.94		7.54	744.0
H. J..........	M.	38	Syphilis	++++	1.16	7.94	2.92	7.51	744.0
M. G..........	F.	66	Syphilitic ulcer of leg	++++	1.16	7.94			
M. D..........	M.	24	Syphilis	++++	1.13	7.95	2.79	7.56	748.5
M. B..........	F.	42	Syphilis	++++	1.13	7.95			
F. C..........	M.	33	Syphilis (many old scars)	negative	1.13	7.95	2.85	7.55	749.6
S. G..........	M.	25	Syphilis (eczema)	++++	1.11	7.96			
W. G. C......	M.	43	Syphilis (pustular)	++++	1.08	7.97			

Alkalinity of the blood in skin disease. Some twenty cases of skin diseases of various kinds were examined, the results being set forth in table 4. These show a good deal more variation than the cases reported in the preceding tables, about 30 per cent only giving normal readings. There are five quite strik-

TABLE 4

NAME	SEX	AGE	CLINICAL DIAGNOSIS	SERUM		BLOOD		BAROMETRIC PRESSURE
				10^{-8}	pH	10^{-8}	pH	
								mm. Hg
M. L.	F.	56	Varicose ulcer of leg	1.22	7.92	3.27	7.49	741.5
F. B.	F.	79	Varicose ulcer of leg	1.52	7.82	3.20	7.50	741.5
A. O. S.	M.	61	Chronic varicose ulcer of leg	1.02	7.99	2.07	7.69	759.7
I. F.	M.	14	Psoriasis, general (extensive)	0.80	8.10			
W. D.	M.	52	Pemphigus, acute (advanced)	1.62	7.79			
L. S.	F.	5	Pemphigus, acute (early)	1.43	7.85			
M. C.*	F.	51	Pemphigus, vegetans	1.30	7.89	2.38	7.63	744.5
C. W.	M.	38	Dermatitis herpetiformis	1.27	7.90	1.89	7.73	761.5
L. J.	M.	43	Eczematoid dermatitis of leg (previous attacks of rheumatism)	1.52	7.82			
J. K.	M.	37	Eczematoid dermatitis, general	1.13	7.95	3.28	7.49	739.5
A. L.	F.	56	Eczema of face and hands	1.16	7.94	2.99	7.53	743.0
W. E. K.	M.	47	Eczema of leg	1.30	7.89	2.73	7.57	745.7
J. M.	M.	51	Eczema of leg	1.24	7.91	1.89	7.73	761.5
B. G.	F.	34	Eczema of leg (indicanuria)	0.98	8.01	2.66	7.58	750.7
D. P.	M.	8	Exudative diathesis	1.02	7.99	3.20	7.50	743.0
W. I.	M.	62	Pityriasis rubra	1.22	7.92	2.54	7.60	753.3
J. M. S.	M.	49	Papilloma of finger	1.16	7.94	2.99	7.53	744.5
A. T.	M.	23	Molluscum fibrosum (general)	0.94	8.03			744.0
W. M. C.	F.	23	Molluscum fibrosum (general)	0.73	8.14	2.66	7.58	750.7
N. D.	F.	49	Lupus erythematosus (face)	1.55	7.81	3.13	7.51	741.5
C. J.	F.	26	Lupus erythematosus (face), January 30	0.88	8.06	3.05	7.52	743.9
			Lupus erythematosus (face), after treatment March 17	1.02	7.99	2.79	7.56	751.2
E. F.	F.	44	Lupus	1.08	7.97	3.13	7.51	741.6
H. R.	M.	16	Acne vulgaris (general)	1.33	7.88	3.06	7.52	740.0

* I am indebted to Dr. M. F. Engman for this case.

ingly above normal which have a value of pH of 8.10, 8.01, 8.14, 8.03 and 8.06. One of these was a case of eczema of the leg, and her urine was marked by a large amount of indican. A similar degree of indicanuria was associated with a high alkalinity of the blood, as in one of the normal cases already referred to. The highest value was found in a case of molluscum fibrosum. In this case, W. M. C., the disease was of twelve years' duration, and the symptoms were marked. The second patient, A. T., had a much lower reading, and the skin nodules were small and few in number, though the onset of the disease dated eight years previously. This high alkalinity is interesting from the fact that this disease is included under new growths, though different authors are divided in opinion as regards its exact etiology. The very low alkalinity in the pemphigus cases should also be mentioned. In the advanced case, W.D., who died a few weeks after the blood examination was made, the value of pH was only 7.79. The early stage, L. S., had also a very low reading of 7.85. In the lupus case with a high alkalinity the value of pH fell from 8.06 to nearly a normal value of 7.99 after treatment and dieting on one of the lactic acid bacilli preparations of milk.

Alkalinity of blood serum in diabetes mellitus. There were eleven cases of diabetes mellitus, the results of the examination being shown in table 5. These all showed a striking and characteristic increase in the alkalinity of the serum. These cases were not complicated by acidosis. It will be noticed that the values ranged from pH 7.99 to 8.35, all but two being above 8.0. One of these two had a very low alkalinity of the serum, namely, a pH of 7.83. As the whole blood in this individual was practically normal, I suspect that this may have been an error of observation. The heart cases mentioned later, which show such an increase in acidity of the serum, very frequently show a change in the alkalinity of the whole blood. It will be noticed how the alkalinity increased in some of these cases after the ingestion of sodium bicarbonate. In the patient M. G., this increased alkalinity of the blood was coincident with a most marked increased rate of tumor growth. After the Allen starvation

TABLE 5

NAME	SEX	AGE	CLINICAL DIAGNOSIS	SERUM		BLOOD		BAROMETRIC PRESSURE
				10^{-8}	pH	10^{-8}	pH	
								mm. Hg
M. D......	M.	45	Diabetes mellitus	1.02	7.99			
E. D.*......	M.	50	Diabetes mellitus	0.99	8.01	2.55	7.60	750.7
M. F........	F.	51	Diabetes mellitus (epithelioma of nose)	0.92	8.04	3.35	7.48	741.0
W. S........	M.	73	Diabetes mellitus (leucoplakia of tongue)	0.88	8.06			
C. P. A.*...	M.	52	Diabetes mellitus (after Allen treatment)	0.47	8.33	2.55	7.60	753.0
J. E. B....	M.	36	Diabetes mellitus (after Allen treatment)	0.45	8.35	2.07	7.69	759.7
M. G. ...	F.	45	Diabetes mellitus (carcinoma of breast)	0.45	8.35	2.92	7.54	749.5
A. C.*....	M.	54	Diabetes mellitus	1.50	7.83	2.67	7.58	750.7
M. G........	F.	57	Diabetes mellitus (endothelioma of face)	0.99	8.01			
			After treatment with $NaHCo_3$	0.64	8.20			
M. R........	F.	64	Diabetes mellitus:					
			February 2, 1916. Dextrose in urine (24 hours), 22.5 grams	0.73	8.14	2.17	7.67	758.5
			February 28, 1916. After treatment with $NaHCO_3$. Dextrose in urine (24 hours), 3.4 grams	0.59	8.23			
			March 4, 1916. Dextrose in urine (24 hours), 22.2 grams	0.67	8.18	2.99	7.53	747.5
			March 30, 1916. Dextrose in urine (24 hours), 96.0 grams	0.84	8.08			
			April 3, 1916. After three days' starvation Allen treatment. Dextrose in urine (24 hours), negative	0.70	8.16			
			April 2, 1916. Dextrose in urine (24 hours), negative	0.55	8.26			
M. R........	F.	47	Diabetes mellitus. Dextrose in urine (24 hours), 157 grams	0.80	8.10			
			Ten days after Allen treatment. Dextrose in urine (24 hours), negative	0.54	8.27	2.92	7.51	749.0
			Seventeen days after Allen treatment. Dextrose in urine (24 hours), trace	0.64	8.20	3.43	7.47	741.0

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* I am indebted for these cases to Dr. F. B. Hall who kindly made arrangements for obtaining them from the Missouri-Pacific Hospital.

treatment there is a marked rise in alkalinity, until dextrose again appears in the urine, when the alkalinity again falls.

Alkalinity in miscellaneous medical cases. Twenty-six miscellaneous medical cases are included in table 6. Most of these proved to have sera which were less alkaline than normal. This is particularly marked in the cases of rheumatism and chronic endocarditis. These cases are interesting from the fact that with the diminished alkalinity of the serum in certain of them there is a corresponding diminution in the alkalinity of the whole blood as evidenced by the position of these values on the lower curve in figure 3. This fact is in agreement with the observations of Peabody (8). By direct electrical measurements this author also found the hydroxyl ion concentration in such cases considerably below the average. Similar conclusions have been reached by many workers using various indirect methods of measuring the blood reaction, among which may be mentioned the work of Poulton and Ryffel (20), and of Lewis, Ryffel, Wolf, Cotton and Barcroft (21). These workers used the dissociation curve of haemoglobin as their index of acidity. They found that the haemoglobin capacity for oxygen was distinctly lowered, a phenomenon which they regarded as due to increased acidity of the blood. However, their analyses showed little or no increase in lactic or any organic acid. Further, the urea, ammonia, and "rest" nitrogen of the blood were not increased. Our results indicate quite clearly that an increase in the acidity of the serum does occur. It may be noted that two of the patients suffering from heart lesions showed an increased instead of a decreased alkalinity.

The case of constipation, A. M., was of considerable interest. She entered the hospital with the diagnosis of cancer of the rectum, but the alkalinity of the blood serum proving to be normal, a more careful examination showed the symptoms to be due to constipation. The cholelithiasis had high alkalinity. In the three cases of leucaemia of which the blood reaction was measured, the two spleno-myelogenous cases gave a high acidity, in contrast to the lymphatic type in which the alkalinity was increased.

TABLE 6

NAME	SEX	AGE	CLINICAL DIAGNOSIS	SERUM		BLOOD		BAROMETRIC PRESSURE
				10^{-8}	pH	10^{-8}	pH	*mm.* Hg
S. O.	F.	39	Cholelithiasis, obstructive. Jaundice, necrosis of liver; serum contains bile	0.82	8.09			
H. G.*	M.	60	Gout (morphinism)	0.55	8.26			
J. H. P.*	M.	23	Paranoia (?)	0.91	8.04	2.07	7.69	759.7
I. R.	F.	38	Grave's disease (mild)	1.08	7.97			
G. E.†	M.	52	Cirrhosis of liver	1.35	7.87			
W. B.†	M.	65	Elephantiasis of leg	1.38	7.86			
M. M.†	F.	70	Elephantiasis of leg	0.91	8.04			
A. M.	F.	69	Constipation	1.16	7.94	1.84	7.74	762.0
H. M. H.*	M.	45	Acute articular rheumatism	1.59	7.80	2.54	7.60	753.4
S. M. A.*	F.	24	Acute articular rheumatism	1.41	7.85	2.66	7.58	753.4
E. O.*	F.	52	Chronic syphilitic endocarditis, myocarditis, aortitis, nephritis—marked dyspnoea (died two weeks subsequently)	1.93	7.72	3.35	7.48	750.0
P. M.*	M.	15	Septic endocarditis—marked dyspnoea (died ten days subsequently)	1.64	7.79	2.73	7.57	750.0
J. F.*	F.	46	Myocarditis with mitral insufficiency (dyspnoea)	1.43	7.85	2.93	7.54	750.0
J. B.*	F.	58	Aneurysm of arch of aorta (dyspnoea absent)	1.30	7.89	2.73	7.57	750.0
H. M. S.*	M.	33	Chronic endocarditis and aortic stenosis	1.35	7.87	2.66	7.58	753.4
R. S.*	M.	68	Auricular fibrillation with hypertension, endocarditis, nephritis	1.54	7.82	2.85	7.55	753.4
P. D.†	M.	61	Chronic nephritis with hypertension	1.91	7.72			
A. L.	F.	41	Chronic nephritis (syphilis)	1.53	7.82			
M. M.†	F.	45	Chronic endocarditis with mitral insufficiency (chronic nephritis)	0.98	8.01			
S. F.†	F.	71	Chronic endocarditis with mitral insufficiency	0.74	8.13			
G. M.*	M.	31	Miliary tuberculosis (pulmonary), alcoholic neuritis (?)	1.29	7.89	2.54	7.60	751.0
C. A.	M.	18	Tuberculosis of cervical glands	1.94	7.72			
H. G.	F.	48	Ventral hernia (obesity)	1.16	7.94	1.93	7.72	762.0
J. M.	M.	39	Lymphatic leucaemia	1.02	7.99	2.99	7.53	760.5
P. I.†	F.	42	Spleno myelogenous leucaemia	1.30	7.89			
J. Q.†	M.	24	Spleno-myelogenous leucaemia	1.70	7.76			

* I am indebted for these cases to Dr. M. F. Engman, who kindly made arrangements for obtaining them from medical wards at Barnes Hospital.

† I am indebted to Dr. N. B. Carson for these cases, which were obtained from his service at Mullanphy Hospital.

Alkalinity of the serum in carcinoma of the stomach and some other stomach diseases. Table 7 contains the results obtained with six cases of carcinoma of the stomach. All these showed a marked increase in alkalinity of the serum, while one case had the very high alkalinity of pH 8.44. The high reading obtained in the sera of the patient M. P. M. is interesting because of the history of the case. An exploratory operation was performed one year previously and a mass about the size of a small orange found at the pyloric end of the stomach. No histological preparations were obtained, but the Wassermann reaction having been negative, a diagnosis of carcinoma was made. Under deep X-ray therapy Dr. Hall was able to reduce the mass so that at the time of the blood examination X-ray pictures showed the stomach normal in outline with the exception of a slight constriction at the pyloric opening. Under treatment the patient had gained 30 pounds in weight. Whether the deformity at the pylorus was due to adhesions or was the remains of the original mass could not be definitely made out. Even with the reduction of the growth the alkalinity of the serum was very high. In addition, several other stomach diseases are included. Of these the gastric neurosis and the uncinariasis were normal. The case of uncinariasis was sent into the hospital with the diagnosis of cancer, but proved not to be. The high figure in ulcer of the stomach is very interesting. The three figures given in the table were obtained while the patient was on various diets, but in each instance no alteration occurred in the value of serum reaction. The last reading was obtained after operation. The case of gastro-enteroptosis was surprisingly high. She had been operated on two years earlier for gall stones. The high alkalinity in the carcinoma cases, to which there was no exception, is the striking fact brought out in this examination.

Alkalinity of the serum in carcinoma of the breast. Five cases of carcinoma of the breast are included in table 8. All showed an increased alkalinity, pH being between 8.01 and 8.35. One case of chronic mastitis had a normal alkalinity; the other case had a high alkalinity. In this as in subsequent tables the duration of the disease can be given only approximately.

TABLE 7

NAME	SEX	AGE	CLINICAL DIAGNOSIS	PATHOLOGICAL DIAGNOSIS	SERUM		BLOOD		BAROMETRIC PRESSURE
					10^{-8}	pH	10^{-8}	pH	
									mm. Hg
E. L	M.	54	Carcinoma of stomach (upper half). Metastases in liver, omentum and intestines	Adeno carcinoma involving liver, intestines and omentum	0.95	8.02	2.07	7.69	760.0
J. W	M.		Carcinoma of stomach	Adeno carcinoma of stomach	0.87	8.06			
L. C.	M.	59	Carcinoma of stomach (pylorus)	Adeno carcinoma of stomach	0.69	8.16			
H. M.	M.	36	Carcinoma of stomach (pylorus)	Adeno carcinoma of stomach	0.65	8.19			
M. P. M.*	M.	36	Carcinoma of stomach (pylorus)	Adeno carcinoma of stomach	0.63	8.20	2.92	7.54	749.2
F. R.	F.	42	Carcinoma of stomach (pylorus)	Inoperable	0.36	8.44			
A. P.	F.	23	Gastric neurosis		1.25	7.90			
J. P.	M.	64	Uncinariasis		1.25	7.90			
B. P.	F.	31	Gastric neurosis		1.14	7.94	2.60	7.59	749.6
F. W.	M.	45	Ulcer of stomach at pyloric ring	Ulcer of stomach					
November 3, 1915					1.00	8.00			
November 24, 1915					1.00	8.00			
January 4, 1916					0.99	8.01	2.72	7.57	745.0
J. R.	F.	22	Gastro-enteroptosis	X-Ray examination. Hourglass contraction of stomach. Adhesions about appendix	0.62	8.21	2.55	7.60	753.3

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[*] I am indebted to Dr. F. Hall for this case.

TABLE 8

NAME	AGE	CLINICAL DIAGNOSIS	PATHOLOGICAL DIAGNOSIS	DURATION OF DISEASE	SERUM		BLOOD		BAROMETRIC PRESSURE
					10^{-8}	pH	10^{-8}	pH	
									mm. Hg
E. D......	33	Carcinoma of breast and head	Squamous cell epithelioma of scalp. Scirrhous carcinoma of breast	Three years	0.98	8.01			
K. W.	57	Carcinoma of breast and glands	Adeno carcinoma of breast	Two years	0.94	8.03	3.20	7.50	711.5
A. H.	43	Carcinoma of breast and glands	Inoperable	Six months	0.86	8.07			
M. M. ...	52	Carcinoma of breast and glands	Carcinoma of breast	One year	0.75	8.13			
S. G.*	45	Carcinoma of breast (diabetes mellitus)	Carcinoma of breast	One year	0.45	8.35	2.92	7.54	749.5
P. B......	20	Chronic mastitis (mastodynia)	Cystic mastitis		1.11	7.96			
E. L. B...	55	Chronic cystic mastitis	Cystic mastitis		0.82	8.09	1.93	7.72	758.7

[*] I am indebted to Dr. N. B. Carson for this case.

TABLE 9

NAME	AGE	CLINICAL DIAGNOSIS	PATHOLOGICAL DIAGNOSIS	DURATION OF DISEASE*	SERUM		BLOOD		BAROMETRIC PRESSURE
					10^{-8}	pH	10^{-8}	pH	
									mm. Hg
N. L.	51	Recurrent carcinoma of vagina (uterus removed one year previously)	Carcinoma	One and a half years	0.98	8.01	2.72	7.57	749.6
K. P.	54	Carcinoma of abdomen (uterus removed five years previously)	Carcinoma	Ten years	0.92	8.04	1.93	7.72	758.7
M. Y.	28	Carcinoma of uterus	Carcinoma	Fifteen months	0.92	8.06	2.92	7.54	739.0
L. K.	60	Carcinoma of vulva and glands	Carcinoma of vulva	Number of years	0.88	8.06			
S. W.	60	Carcinoma of uterus	Inoperable	Two years	0.85	8.08			
G. C.	18	Carcinoma of cervix	Carcinoma of cervix	Six months	0.85	8.08			
E. T.	60	Carcinoma of vulva	Carcinoma of vulva	Eighteen months	0.85	8.08			
C. P.	60	Carcinoma of uterus	Inoperable	One year	0.85	8.08	3.05	7.52	742.5
S. M.	62	Carcinoma of uterus	Carcinoma of uterus	Three years	0.85	8.08			
B. F.	32	Carcinoma of cervix	Carcinoma of cervix	Eighteen months	0.82	8.09	1.93	7.72	750.7
J. G.	51	Carcinoma of uterus	Carcinoma of uterus	One year	0.82	8.09			
E. S.	58	Carcinoma of vagina	Carcinoma of vagina	Two years	0.73	8.14	2.02	7.70	758.5
M. D.	65	Carcinoma of vulva and glands	Epithelioma of vulva	Two years	0.72	8.15	2.27	7.65	756.5
E. T.	24	Pregnancy (fourth month), cystic ovary			1.35	7.87	3.20	7.50	743.5
B. N.	42	Myoma of uterus (pyosalpinx)	Myoma (pyosalpinx)		1.02	7.99	2.02	7.70	758.4
E. T.	46	Myoma of uterus (pyosalpinx)	Myoma		0.95	8.02	2.17	7.68	758.5
P. C.	38	Myoma of uterus (pyosalpinx)	Myoma (pyosalpinx)		0.91	8.04	2.37	7.63	753.0

* Periods of time given are only approximate.

TABLE 10

NAME	SEX	AGE	CLINICAL DIAGNOSIS	PATHOLOGICAL DIAGNOSIS	DURATION OF DISEASE	SERUM		BLOOD		BAROMETRIC PRESSURE
						10^{-8}	pH	10^{-8}	pH	
										mm. Hg
H. R.*......	M.	35	Sarcoma of shoulder			0.45	8.35	2.85	7.55	749.2
C. J.........	M.	54	Sarcoma of shoulder	Round cell sarcoma		0.46	8.34	3.13	7.51	743.0
H. L.........	M.	56	Carcinoma of rectum	Adeno carcinoma	One year	0.78	8.11	2.02	7.70	759.3
A. J. B......	M.	52	Carcinoma of caecum	Carcinoma		0.78	8.11			
M. C.........	M.		Carcinoma of intestine, liver and kidney	Inoperable		0.75	8.13			
W. B.*......	M.		Carcinoma of oesophagus	Inoperable		0.84	8.08	2.92	7.54	745.7
G. M.........	M.		Carcinoma of stomach, liver and intestines	Inoperable		0.83	8.09	2.60	7.59	750.0
M. P.........	M.	64	Carcinoma of prostate	Carcinoma of prostate	One year	0.91	8.04			
L. T.†......	F.		Carcinoma of pancreas	Carcinoma of pancreas	One year	0.72	8.15			
E. B.........	F.	22	Carcinoma of sigmoid flexure	Carcinoma		1.03	7.99			
G. R. S.*...	M.		Carcinoma of sigmoid flexure	Carcinoma		1.15	7.94	2.60	7.59	750.7
M. J.........	M.	64	Synovitis	Xanthoma of knee	Twenty years	1.21	7.92	2.85	7.55	749.6
G. K......	M.	50	Sarcoma, eye; metastases in lungs, intestines, etc.	Inoperable sarcoma of eye, with metastases in lungs, intestines, etc.	Some years	1.11	7.96			

* I am indebted to Dr. F. Hall for the above cases.

† I am indebted to Dr. H. N. Lyon for this case.

TABLE 11

NAME	SEX	AGE	CLINICAL DIAGNOSIS	PATHOLOGICAL DIAGNOSIS	APPROXIMATE DURATION OF DISEASE	SERUM		BLOOD		BAROMETRIC PRESSURE
						10^{-8}	pH	10^{-8}	pH	
										mm. Hg
T. M.	M.	61	Epithelioma of lip	Basal cell carcinoma—superficial. No invasion of muscle	Five months	1.30	7.89			
F. B.	M.	68	Epithelioma of lip	Epithelioma (early)	Eight years	1.16	7.94			
R. B.	M.	91	Epithelioma of lip	Epithelioma	Two years	1.11	7.96			
R. S.		65	Epithelioma of lip			1.08	7.97			
W. S. G.	M.	17	Epithelioma of lip		One year	1.08	7.97			
J. J. D.	M.	65	Epithelioma of lip	Epithelioma	Three years	1.16	7.94	1.84	7.74	762.0
M. D.	M.	65	Epithelioma of lip	Epithelioma	Three years	0.99	8.01			
J. D	M.	63	Epithelioma of lip	Epithelioma	Three months	0.92	8.04			
W. M.	M.	60	Epithelioma of lip		Six years	1.00	8.00			
T. C.	M.	50	Epithelioma of lip (mucous surface)	Epithelioma	Nine months	0.85	8.08	2.55	7.60	753.0
L. J.	M.	61	Epithelioma of face	Epithelioma	Three years	1.00	8.00			
L. H.	M.	68	Epithelioma of nose	Basal cell carcinoma	Six years	0.96	8.02	2.60	7.59	751.0
N. B.	M.	62	Epithelioma of nose		(?)	0.92	8.04	2.07	7.69	758.4
O. P.	M.	70	Recurrent epithelioma of nose	Epithelioma	Twenty-five years	0.90	8.05			
J. S.	F.	40	Recurrent epithelioma of nose	Epithelioma		0.88	8.06			
C. K.	M.	50	Epithelioma of nose and cheek		Nine years	0.99	8.01			
J. A. H.	M.	65	Epithelioma of nose and cheek	Inoperable	Twenty-five years	0.88	8.06	3.35	7.48	739.5
J. A. C.	M.	74	Epithelioma of cheek		Seven years	0.96	8.02			

W. S.	M.	73	Epithelioma of cheek (diabetes)	Leucoplakia	Ten years	0.88	8.06			
J. B.	M.	53	Epithelioma of mouth		Seven years	0.86	8.07	2.49	7.61	751.0
S. B. M.	M.	68	Epithelioma of mouth	Epithelioma	Seven years	0.88	8.06			
J. F.	M.	71	Epithelioma of jaw	Epithelioma	Three months	1.00	8.00			
C. C. W.	M.	67	Epithelioma of jaw	Epithelioma	Two years	1.00	8.00			
L. P.	F.	52	Epithelioma of jaw	Epithelioma		0.99	8.01			
E. W.	M.	81	Epithelioma of jaw	Epithelioma	Number of years	0.99	8.01			
A. M. D.	M.	53	Epithelioma of jaw	Epithelioma	Two years	0.82	8.09			
H. M.	M.	50	Epithelioma of jaw involving mouth	Epithelioma	One year	1.02	7.99			
T. D.	M.	66	Epithelioma of neck	Epithelioma	Four years	0.95	8.02			
W. E. W.	M.	53	Epithelioma of neck	Carcinoma (superficial)	Twelve years	0.94	8.03			
A. C.	M.	77	Epithelioma of umbilicus	Papilloma	One year	1.11	7.96			
J. M.	M.	40	Epithelioma of eyelid	Epithelioma	One year	0.99	8.01			
W. H.	M.	63	Epithelioma of eyelid	Epithelioma	Three years	0.95	8.02			
M. B.	M.	56	Epithelioma of eyelid	Epithelioma	Nine years	0.88	8.06			
H. E.	M.	40	Epithelioma of forehead		Six months	0.99	8.01			
J. G.	M.	50	Epithelioma of forehead		Four months	0.88	8.06	2.42	7.62	753.5
R. K.	F.	35	Epithelioma of labia majora	Squamous epithelioma of vulva	Two years	1.08	7.97			
E. D.	F.	35	Epithelioma of scalp and breast	Squamous epithelioma of head. Scirrhous carcinoma of breast	Three years	0.99	8.01	3.20	7.50	741.5
M. G.	F.	57	Epithelioma of face (diabetes)	Endothelioma (malignant)		0.99	8.01			

Alkalinity of serum in carcinoma of the uterus and vagina. Thirteen cases of carcinoma of the uterus and vagina are shown in table 9. In all these the alkalinity of the serum was well above the normal, ranging from 8.01 to 8.15. The three last cases of myoma are also higher than the normal. The case of pregnancy with a cystic ovary had a low alkalinity, lower than the normal of pH 7.94. In two of the patients, aged nineteen and thirty-two years, syphilis was a complication.

Alkalinity of the blood in carcinomas in various organs. The first nine cases in table 10, including sarcomas and carcinomas of various organs, all have a very high alkalinity of the serum, varying from 8.04 to 8.35. Of the two carcinomas of the sigmoid flexure, one was normal; the other was high, but not so high as is usual in cancer. The case of synovitis had to do with a non-malignant neoplasm and the serum in this case was normal in alkalinity. The last case recorded, sarcoma of the eye, was a very advanced case with metastases everywhere, and the patient died soon after the readings were taken. The alkalinity of the blood in this case was about normal. It is possible the very marked cachexia may have reduced the alkalinity of this case, but of course this cannot be definitely stated without further observations.

Alkalinity of the serum in cases of superficial cancer. There are thirty-seven cases of superficial cancer reported in table 11, but three of these have been reported in the other tables. While these do not average as high an alkalinity as the cases reported in cancer of the internal organs, yet nearly all of them are definitely above the normal alkalinity, and many of them have an alkalinity of pH 8.06. The lowest average was found in cancers of the lip, two of which gave normal values. All of these cases give histories of irritation from pipe. It is noteworthy that almost all the superficial cancers studied occur in men, and an examination of the records of cases previously admitted to the hospital shows this same high percentage. The papilloma of the umbilicus was probably not malignant and the alkalinity was normal. On the whole, these skin cancers, with a few exceptions, show the rise in alkalinity of the serum which has been remarked in internal cancer.

Relationship between red blood corpuscles and alkalinity of the serum. As there is frequently present in advanced cases of carcinoma an anaemia of marked degree, blood counts were made on a number of patients to determine whether any relationship existed between the number of erythrocytes and the alkalinity of the serum. The counts were made with the Thoma-Zeiss counting chamber and Toisson's solution was used as a diluent. There was no discernible relationship as is evidenced by the figures in the appended table 12.

TABLE 12

NAME	CLINICAL DIAGNOSIS	SERUM		NUMBER OF ERYTHROCYTES PER MM $^{-3}$
		10^{-8}	pH	
E. O.	Endocarditis (nephritis)	1.93	7.72	4,460,000*
P. M.	Septic endocarditis	1.64	7.79	4,096,000*
R. S.	Endocarditis (nephritis)	1.51	7.82	3,792,000*
L. S.	Pemphigus	1.43	7.85	4,965,710
E. L. L.	Normal (indicanuria)	0.96	8.02	4,260,980
M. Y.	Carcinoma of uterus	0.88	8.06	3,280,320
J. S.	Carcinoma of nose	0.88	8.06	4,760,920
J. G.	Carcinoma of uterus	0.82	8.09	4,832,694
J. F.	Psoriasis	0.80	8.10	6,504,000
H. L.	Carcinoma of rectum	0.78	8.11	2,480,615
A. R.	Diabetes mellitus	0.73	8.14	5,568,300
E. S.	Carcinoma of uterus	0.73	8.14	2,876,815

* These three blood counts were taken from the Barnes Hospital records.

CONCLUSIONS

The general result of this study has been then to show that in nearly all the cases of cancer or sarcoma examined, there is to be found an alkalinity of the serum greater than that of the serum of normal individuals. Of the seventy cases examined, only three or four were found which had an alkalinity of the normal amount or less than normal. In all other cases there was some excess of alkalinity and in some of the cancers of the internal organs this was very marked. In one case in which bicarbonate was given there was also a very marked increase in rate of growth of the cancer. This rise of alkalinity in the blood, while generally occurring in cancer, is not specific for cancer. It was observed in several other conditions. On the other hand, in certain heart lesions with dyspnoea and various other non-malignant diseases, the alkalinity is reduced below the normal. In diabetes mellitus, in the state before acidosis, there is an abnormally high alkalinity. High alkalinity is not, therefore, a certain indication of cancer, but its presence in suspected cancer is an added reason for a positive diagnosis. The examination of the alkalinity of the serum may, therefore, be of considerable practical assistance in the diagnosis of this disease.

We cannot without a more extensive investigation determine whether this increased alkalinity is a causative factor in the disease, or whether it is an accompanying phenomenon. Several facts make us suspect that the former may prove to be the case, and we shall continue this study with the hope that this point, so important for the understanding of the etiology of the disease, may be cleared up.

Throughout the course of this work I have been greatly indebted to many members of the staff of the Barnard Free Skin and Cancer Hospital and especially to Dr. George M. Smith, the former Director of the Hospital and Laboratories, for his most generous support and assistance. Without his aid, much of the work could not have been done.

It is also a pleasure to express my grateful appreciation of the help given by Dr. R. A. McGarry and Dr. R. F. Fisher of the resident staff. My sincere thanks are due Prof. A. P. Mathews of the University of Chicago for suggestions and criticisms in the preparation of the manuscript, and to Prof. J. Erlanger of Washington University for the loan of apparatus.

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THE INHERITANCE BEHAVIOR OF INFECTIONS COMMON TO MICE[1]

STUDIES IN THE INCIDENCE AND INHERITABILITY OF SPONTANEOUS TUMORS IN MICE

Ninth Report

MAUD SLYE

From the Cancer Laboratory of the Otho S. A. Sprague Memorial Institute and the University of Chicago

Previous reports from this laboratory during the past six years have demonstrated the inheritability of spontaneous cancer in mice, both of cancer in general and of tumors of specific organs and of specific types. Some commentators have inferred, however, that the fact that the cancer tendency is inheritable has no particular bearing upon the etiology of cancer since they suppose[2] that a study of any of the common infections, such as pulmonary infections, would show similar results.

The full import of such a demonstration and its unquestionable bearing upon the nature of cancer, therefore, stands sharply out when this inheritance behavior of cancer is compared with the carefully tested inheritance behavior of the infections common to mice.

The cancers reported from this laboratory are all spontaneous, arising in the ordinary routine of mouse life without any artificial manipulation of any sort whatever. The infections reported in this paper occur also without any artificial manipulation.

It is the unbroken rule throughout these experiments to let natural causes determine the incidence of both cancer and infections without any interference except that of a rigorous hygiene and of a definite plan of selective breeding uniformly applied throughout the entire stock, both cancerous and non-cancerous. This method of study gives a fair basis for a comparison of the facts of the inheritance behavior of these types of disease.

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[1] Presented before the American Society for Cancer Research, New York, April 5, 1917.

[2] Erwin F. Smith, Further evidence that crown gall of plants is cancer, Science, 1916, xliii, 874.

It must be emphasized in the study of this comparison that there is a tremendous handicap in favor of the occurrence of infections, as follows:

1. Infections may occur at any period in mouse life from the day of birth to the date of death, with the highest incidence during the periods of infancy and of adolescence. On the other hand, cancer rarely occurs under six months in mice, and the high level of incidence centers around the period from ten to twenty months. Hundreds of mice, consequently, die within the infection age, but under the cancer limit; and there is no difficulty whatever in securing strains peppered with infections, while it is tremendously difficult to carry out a cancer strain.

2. A whole laboratory of mice can be swept off within a week by contact contraction of virulent infections, so that a constant warfare against such disease is necessary in order to preserve any stock at all. It is therefore almost impossible to keep any strain free from these infections through contact contraction, so as to be able to make any kind of a study of their inheritance behavior. Whereas, contagion experiments already described in previous publications, which have been consistently carried on in this laboratory for years, have failed to produce one case of contact contraction of cancer.

It is impossible to make any report of the behavior of tuberculosis[3] in mice because mice do not have tuberculosis. In order, however, to give every advantage to the infections in a comparison of their inheritance behavior with that of cancer, I have grouped all pulmonary infections, all intestinal infections, all uterine infections, etc., to which mice are susceptible. The test therefore, it is evident, is a test of the tendency of the intestines, or of the lung, or of the uterus, etc., to yield to specific infections as compared with the inheritability of their tendency to develop cancer.

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[3] Erwin F. Smith, loc. cit.

In spite of the tremendous handicap in favor of the common infections peppering every strain of mice, and in spite of the great difficulty in carrying out one cancer strain to any considerable number, note the results.[4]

CHART 1

Strain 139

In this strain both parents had carcinoma of the lung, male 193, primary carcinoma, and female 158, metastatic carcinoma of the lung. The resulting strain carried 90 per cent of cancer, every member but two having primary tumors of the lung. One member, male 553, died at six months, which is under the age for the occurrence of lung tumors.

In sharp contrast with these results note the inheritance behavior of pulmonary infection shown in strain 20.

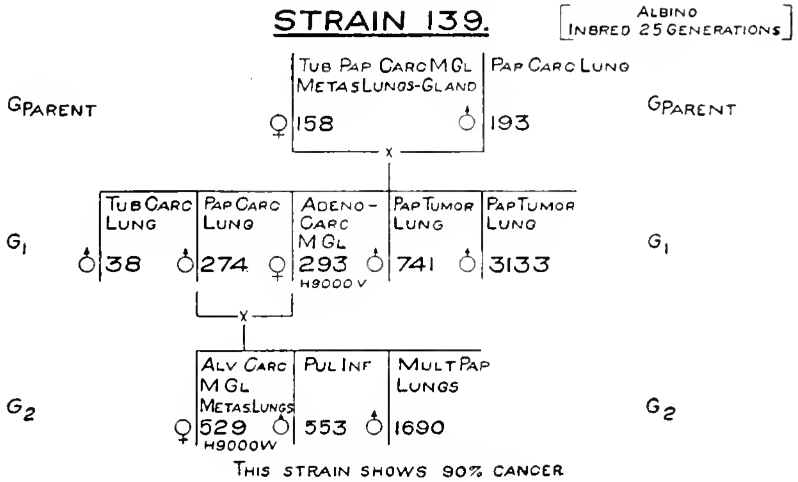

STRAIN 139.
Albino Inbred 25 Generations
Gparent
Tub Pap Carc M Gl Metas Lungs-Gland
♀ 158
♂ 193
Pap Carc Lung
Gparent
x
G1
Tub Carc Lung ♂ 38
Pap Carc Lung ♂ 274
Adeno-Carc M Gl ♀ 293 H9000 V
Pap Tumor Lung ♂ 741
Pap Tumor Lung ♂ 3133
G1
x
G2
Alv Carc M Gl Metas Lungs ♀ 529 H9000W
Pul Inf ♂ 553
Mult Pap Lungs ♂ 1690
G2
This strain shows 90% cancer

Chart 1

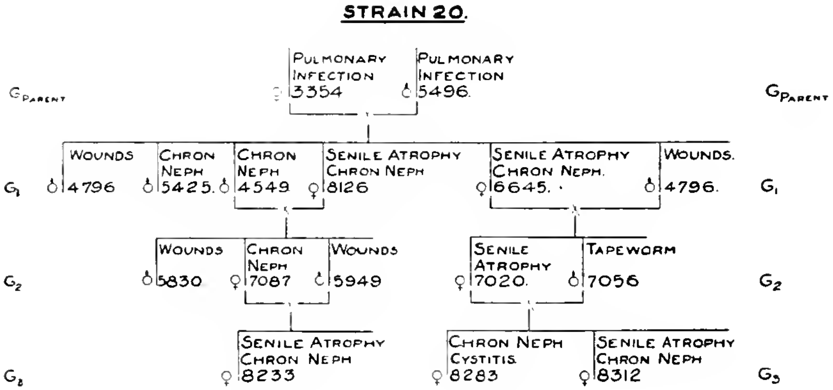
---

[4] Some of these charts have been published previously in whole or in part. They are here repeated for convenience in comparison.

CHART 2

Strain 20

This is a non-cancerous strain of the Japanese waltzing mouse in my hands several years. Although the strain is now living in the eighth generation, it has never shown an occurrence of cancer. In this strain, where both parents died of pulmonary infection, viz., female 3354 and male 5496, there has never been one recurrence of pulmonary infection. This is a striking example of the non-inheritability of pulmonary infection. The same contrast between the inheritance behavior of cancer and that of pulmonary infections is again shown in chart 3.


STRAIN 20.
G PARENT
Pulmonary Infection ♀ 3354
Pulmonary Infection ♂ 5496.
G PARENT
G1
Wounds ♂ 4796
Chron Neph ♂ 5425.
Chron Neph ♂ 4549
Senile Atrophy Chron Neph ♀ 8126
Senile Atrophy Chron Neph. ♀ 6645.
Wounds. ♂ 4796.
G1
G2
Wounds ♂ 5830
Chron Neph ♀ 7087
Wounds ♂ 5949
Senile Atrophy ♀ 7020.
Tapeworm ♂ 7056
G2
G3
Senile Atrophy Chron Neph ♀ 8233
Chron Neph Cystitis ♀ 8283
Senile Atrophy Chron Neph ♀ 8312
G3

A non-cancerous strain of Japanese Waltzing Mice
Both parents died of pulmonary infection
The resulting strain shows 0% pulmonary infection

Chart 2

CHART 3

Strains 280 and 281

In strain 280 both parents had carcinoma of the lung, viz., male 363 and female 258. The resulting family showed 100 per cent of cancer, 80 per cent being tumors of the lung, primary or secondary.

Whereas, in strain 281, although in the first filial generation female 3270 had pulmonary infection, and in another branch of the family in the second filial generation the parent male 2389 died of pulmonary infection, the offspring of these two mice and their respective mates never showed a recurrence of pulmonary infection.

In this same strain where both parents died of tumors, viz., female 258 with carcinoma of the lung and male 286 with adenoma of the liver, the resulting offspring showed about 20 per cent of tumors, 40 per cent of these tumors being located in the liver, and the remaining tumors being located in the lung.

CHART 4

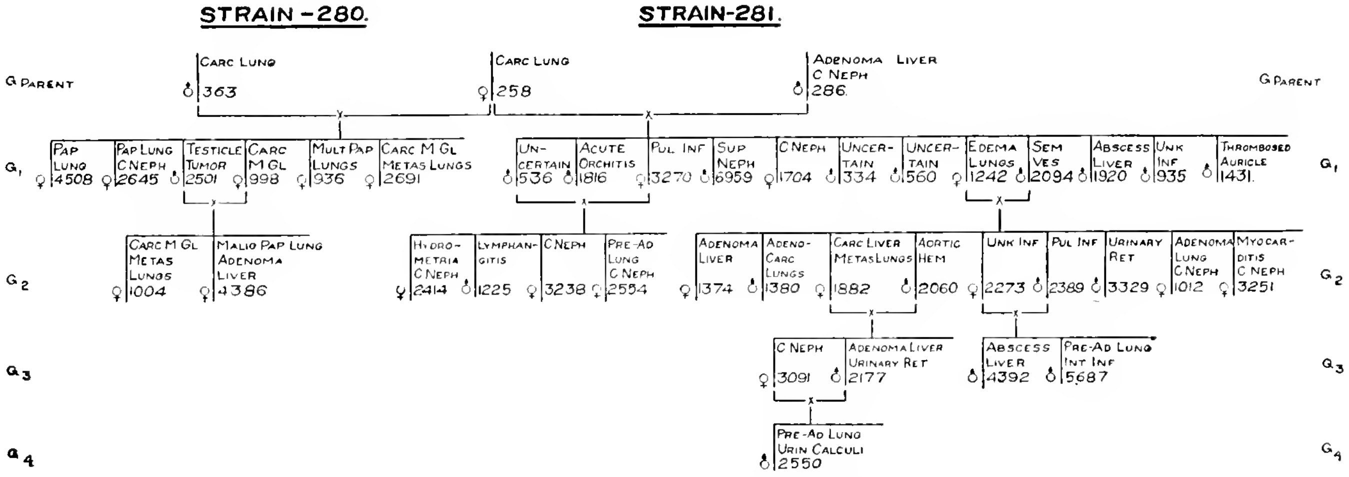
Strain 343

In this strain the parent female 5357 died of carcinoma of the mammary gland, and the parent male 2098 died of pulmonary infection. The resulting strain shows over 58 per cent of cancer, but not one recurrence of pulmonary infection. Here where the two diseases had equal opportunity to be transmitted if both were inheritable, cancer occurred in 58 per cent of the strain and pulmonary infection not at all.

CHART 5

Strain 164. Branch IV

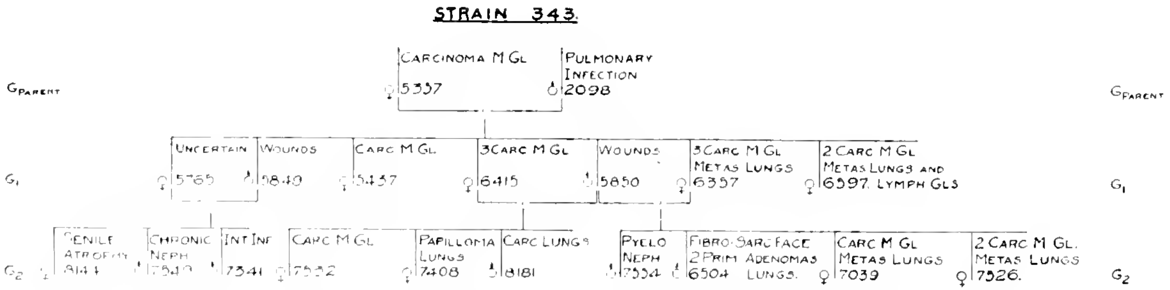
This strain is the result of hybridizing the highly tumorous strain 146 with a non-tumorous strain of house mice, strain 358. Although the parent female, 1236, died early without showing cancer, she evidently transmitted it for the resulting strain shows 24 per cent of tumor. Again, although the parent male in the first filial generation, male 4378, died of pulmonary infection, there was never a recurrence of pulmonary infection throughout the strain, although the family is bred out through five generations involving fifty members. This is another striking case of contrast in the inheritance behavior of cancer and that of pulmonary infection.


STRAIN-280.
STRAIN-281.
G PARENT
G1
G2
G3
G4
♂ 363 CARC LUNG
♀ 258 CARC LUNG
♂ 286 ADENOMA LIVER C NEPH
♀ 4508 PAP LUNG
♀ 2645 PAP LUNG C NEPH
♂ 2501 TESTICLE TUMOR
♀ 998 CARC M GL
♀ 936 MULT PAP LUNGS
♀ 2691 CARC M GL METAS LUNGS
♂ 536 UN-CERTAIN
♂ 1816 ACUTE ORCHITIS
♀ 3270 PUL INF
♂ 6959 SUP NEPH
♀ 1704 C NEPH
♂ 334 UNCER-TAIN
♂ 560 UNCER-TAIN
♀ 1242 EDEMA LUNGS
♂ 2094 SEM VES
♂ 1920 ABSCESS LIVER
♂ 935 UNK INF
♂ 1431 THROMBOSED AURICLE
♀ 1004 CARC M GL METAS LUNGS
♀ 4386 MALIG PAP LUNG ADENOMA LIVER
♀ 2414 HYDRO-METRIA C NEPH
♂ 1225 LYMPHAN-GITIS
♀ 3238 C NEPH
♀ 2554 PRE-AD LUNG C NEPH
♀ 1374 ADENOMA LIVER
♂ 1380 ADENO-CARC LUNGS
♀ 1882 CARC LIVER METAS LUNGS
♂ 2060 AORTIC HEM
♀ 2273 UNK INF
♂ 2389 PUL INF
♂ 3329 URINARY RET
♀ 1012 ADENOMA LUNG C NEPH
♀ 3251 MYOCAR-DITIS C NEPH
♀ 3091 C NEPH
♂ 2177 ADENOMA LIVER URINARY RET
♂ 4392 ABSCESS LIVER
♂ 5687 PRE-AD LUNG INT INF
♂ 2550 PRE-AD LUNG URIN CALCULI
X

STRAIN 280 100% TUMOR
80% HAD LUNG TUMOR PRIMARY OR SECONDARY

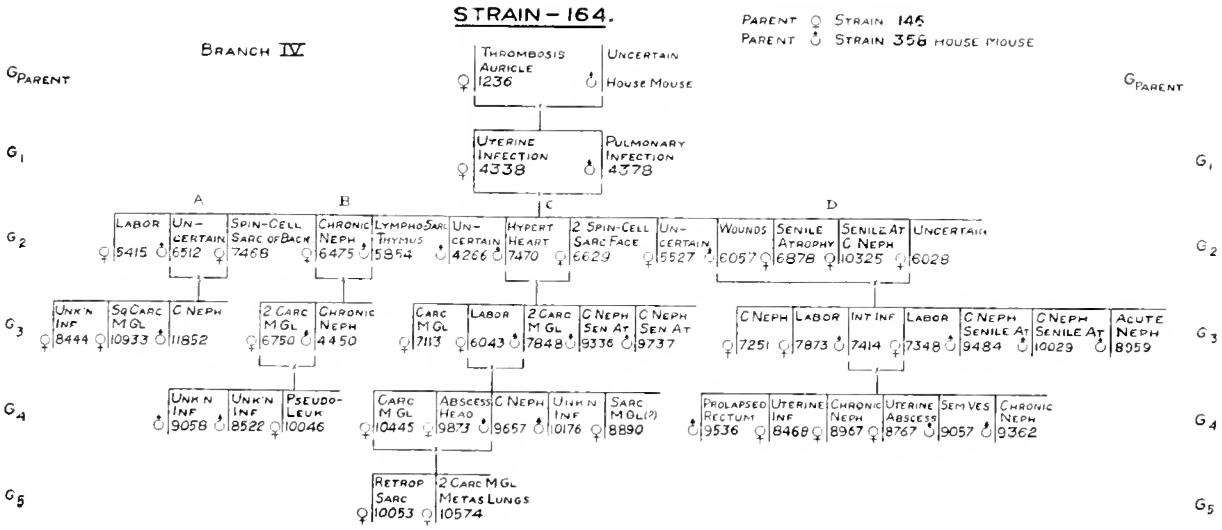
STRAIN 281 NEARLY 20% TUMOR
60% OF THE TUMOR WERE OF LIVER

CHART 3


STRAIN 343.
G PARENT
♀ Carcinoma M Gl 5337
♂ Pulmonary Infection 2098
G1
♀ Uncertain 5765
♂ Wounds 5840
♀ Carc M Gl 5457
♀ 3 Carc M Gl 6415
♂ Wounds 5850
♀ 3 Carc M Gl Metas Lungs 6357
♀ 2 Carc M Gl Metas Lungs and 6597. Lymph Gls
G2
♀ Senile Atrophy 8144
♂ Chronic Neph 7549
♂ Int Inf 7341
♀ Carc M Gl 7552
♀ Papilloma Lungs 7408
♂ Carc Lungs 8181
♂ Pyelo Neph 7554
♂ Fibro-Sarc Face 2 Prim Adenomas Lungs. 6504
♀ Carc M Gl Metas Lungs 7039
♀ 2 Carc M Gl. Metas Lungs 7526.

Parent female - cancer
Parent male - pulmonary infection
The resulting strain shows {over 38% cancer
0% pulmonary infection

CHART 4


STRAIN-164.
BRANCH IV
PARENT ♀ STRAIN 145
PARENT ♂ STRAIN 356 HOUSE MOUSE
G PARENT
G1
G2
G3
G4
G5
♀ THROMBOSIS AURICLE 1236
♂ UNCERTAIN HOUSE MOUSE
♀ UTERINE INFECTION 4338
♂ PULMONARY INFECTION 4378
A
B
C
D
♀ LABOR 5415
♂ UN-CERTAIN 6512
♀ SPIN-CELL SARC OF BACK 7468
♀ CHRONIC NEPH 6475
♂ LYMPHO SARC THYMUS 5854
♂ UN-CERTAIN 4266
♂ HYPERT HEART 7470
♀ 2 SPIN-CELL SARC FACE 6629
♀ UN-CERTAIN 5527
♂ WOUNDS 6057
♀ SENILE ATROPHY 6878
♀ SENILE AT C NEPH 10325
♀ UNCERTAIN 6028
♀ UNK'N INF 8444
♀ SQ CARC M GL 10933
♂ C NEPH 11852
♀ 2 CARC M GL 6750
♂ CHRONIC NEPH 4450
♀ CARC M GL 7113
♀ LABOR 6043
♂ 2 CARC M GL 7848
♂ C NEPH SEN AT 9336
♂ C NEPH SEN AT 9737
♀ C NEPH 7251
♀ LABOR 7873
♂ INT INF 7414
♀ LABOR 7348
♂ C NEPH SENILE AT 9484
♂ C NEPH SENILE AT 10029
♂ ACUTE NEPH 8959
♂ UNK'N INF 9058
♂ UNK'N INF 8522
♀ PSEUDO-LEUK 10046
♀ CARC M GL 10445
♀ ABSCESS HEAD 9873
♂ C NEPH 9657
♂ UNK'N INF 10176
♀ SARC M GL(?) 8890
♂ PROLAPSED RECTUM 9536
♀ UTERINE INF 8468
♀ CHRONIC NEPH 8967
♀ UTERINE ABSCESS 8767
♂ SEM VES 9057
♂ CHRONIC NEPH 9362
♀ RETROP SARC 10053
♀ 2 CARC M GL METAS LUNGS 10574

THIS BRANCH OF THE STRAIN STILL SHOWS 24% TUMOR

CHART 5

CHART 6

Strain 151

This strain furnishes another marked instance of the failure of pulmonary infection to be introduced into the strain where one of the parents died of this infection, viz., male 250. This strain carried through four generations never produced another case of pulmonary infection. It carried, however, a low per cent of cancer.

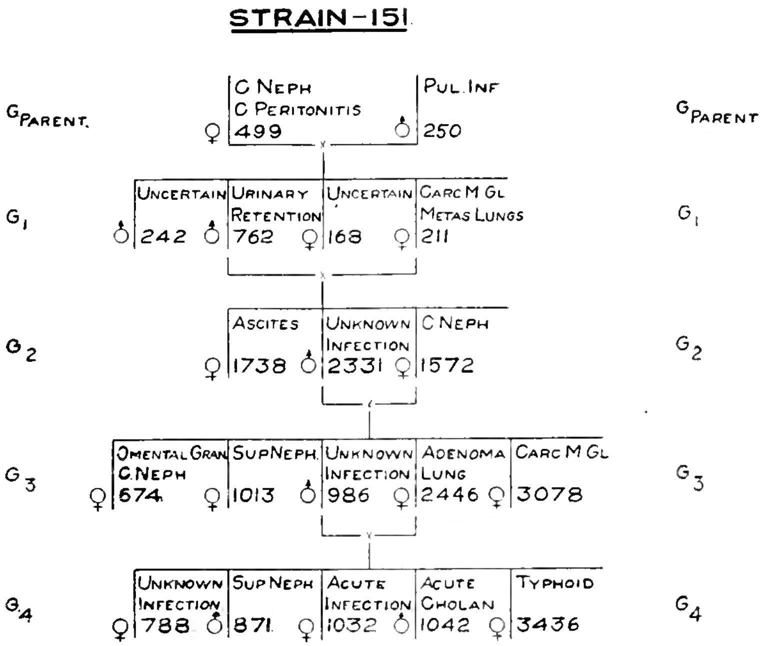

STRAIN-151
G PARENT
♀ 499 C Neph C Peritonitis
♂ 250 Pul. Inf
G PARENT
G1
♂ 242 Uncertain
♂ 762 Urinary Retention
♀ 168 Uncertain
♀ 211 Carc M Gl Metas Lungs
G1
G2
♀ 1738 Ascites
♂ 2331 Unknown Infection
♀ 1572 C Neph
G2
G3
♀ 674 Omental Gran C.Neph
♀ 1013 Sup Neph.
♂ 986 Unknown Infection
♀ 2446 Adenoma Lung
♀ 3078 Carc M Gl
G3
G4
♀ 788 Unknown Infection
♂ 871 Sup Neph
♀ 1032 Acute Infection
♂ 1042 Acute Cholan
♀ 3436 Typhoid
G4

CHART 6

CHART 7

Strain 143

In this strain, also, the parent male 809 in the third filial generation died of pulmonary infection. Here again, there was never another case of pulmonary infection.

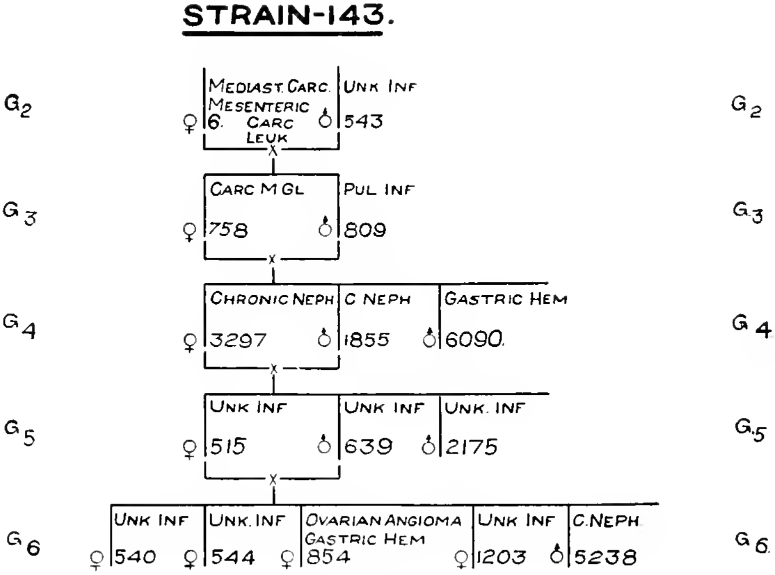

STRAIN-143.
G2
Mediast. Carc.
Mesenteric Carc
Leuk
♀ 6
Unk Inf
♂ 543
G3
Carc M Gl
♀ 758
Pul Inf
♂ 809
G4
Chronic Neph
♀ 3297
C Neph
♂ 1855
Gastric Hem
♂ 6090.
G5
Unk Inf
♀ 515
Unk Inf
♂ 639
Unk. Inf
♂ 2175
G6
Unk Inf
♀ 540
Unk. Inf
♀ 544
Ovarian Angioma
Gastric Hem
♀ 854
Unk Inf
♀ 1203
C.Neph
♂ 5238

Chart 7

CHART 8

Strain 450 with ancestry

This strain shows a striking contrast in the inheritance behavior of cancer on the one hand and of pulmonary infection on the other. The parent female 5924 died of a primary carcinoma of the mammary gland and a primary carcinoma of the lung. She was the hybrid product of four strains all of which carried carcinoma in direct line and all of which showed pulmonary infection, though not in direct line, except in the case of male 250.

The parent male 5183, who died of a hypertrophied heart and intestinal infection, was the product of the hybridization of three strains all of which carried carcinoma in direct line and all of which exhibited pulmonary infection though with the exception of male 809, not in direct line.

Here, then, cancer and pulmonary infection both occurred in the ancestry on both sides of the family, but the resulting strain,

strain 450, showed over 76 per cent of cancer of the same organs and of the same types exhibited in the ancestry, but not one case of pulmonary infection.

The place of male 250 is shown in his inbred strain in chart 6, parent generation, where again there was no recurrence of pulmonary infection. The place of male 809 is shown in his inbred strain in chart 7, third filial generation, where also there was no recurrence of pulmonary infection.

These two males, 809 and 250, both with pulmonary infection, produced offspring which failed to show one case of pulmonary infection, when tested both by inbreeding (charts 6 and 7) and by hybridization (chart 8) which are the only two tests of heredity; and this in spite of the conspicuous prevalence of pulmonary infection in mice.

Contrast with this the occurrence of 76 per cent of cancer in strain 450 where cancer also was bred in on both sides of the ancestry.

CHART 9

Strain 338. Branch III

Here both parents in the first filial generation, viz., female 7899 and male 6851 died of intestinal infection contracted long after separation from their young. The resulting strain shows not one recurrence of intestinal infection, although it is carried out through five generations. Whereas, cancer, which entered in the parent generation, viz., female 5417 and male 7736 occurred in over 45 per cent of the resulting strain.

CHART 10

Strain 338. Branch I

In this branch of the family in the first filial generation, the parent female 9544 died of a carcinoma of the mammary gland, an adenoma of the liver, and a primary carcinoma of the lung; the parent male 6441, died of intestinal infection. The resulting strain shows 40 per cent of tumors, these tumors, with one exception, being carcinoma of the mammary gland, adenoma

STRAIN - 450 WITH ANCESTRY.

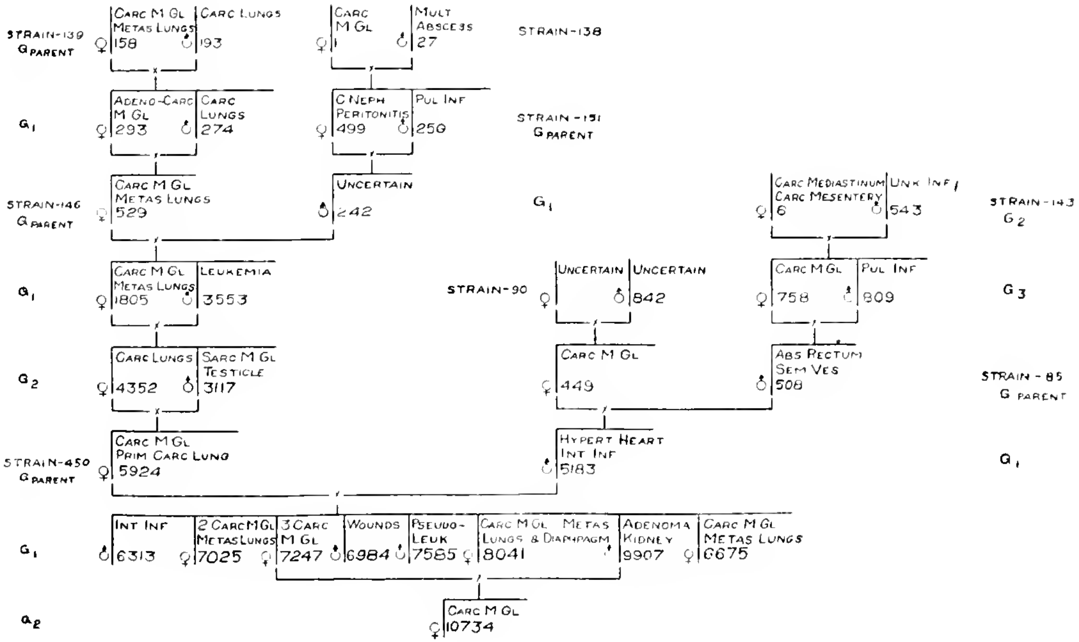
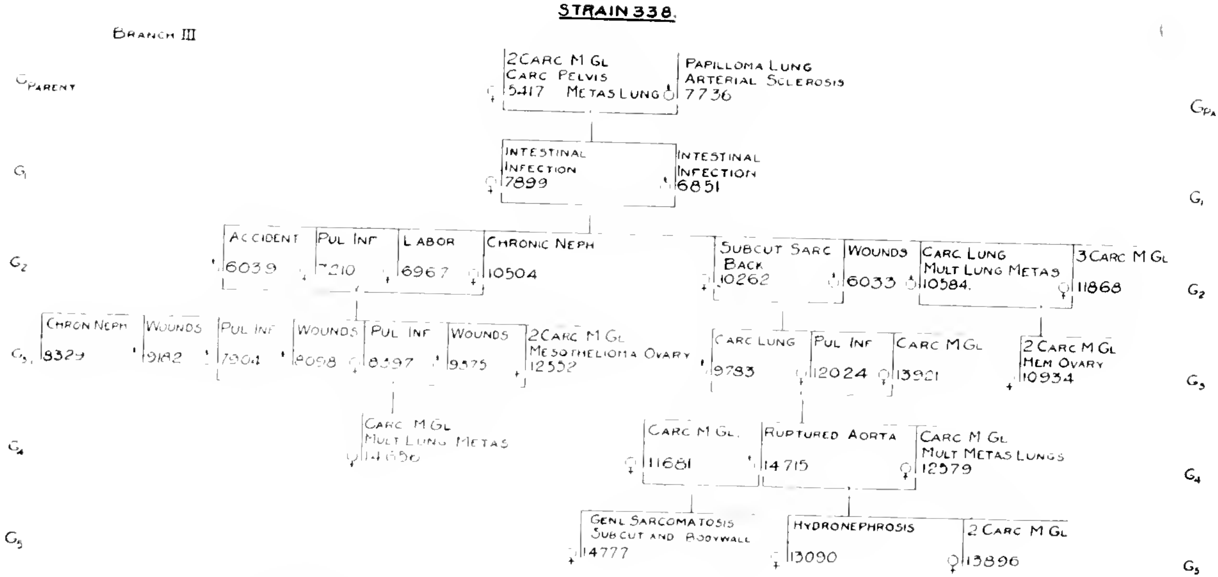

STRAIN-139 G PARENT
Carc M Gl Metas Lungs ♀ 158
Carc Lungs ♂ 193
Carc M Gl ♀ 1
Mult Abscess ♂ 27
STRAIN-138
G1
Adeno-Carc M Gl ♀ 293
Carc Lungs ♂ 274
C Neph Peritonitis ♀ 499
Pul Inf ♂ 256
STRAIN-151 G PARENT
STRAIN-146 G PARENT
Carc M Gl Metas Lungs ♀ 529
Uncertain ♂ 242
G1
Carc Mediastinum Carc Mesentery ♀ 6
Unk Inf ♂ 543
STRAIN-143 G2
G1
Carc M Gl Metas Lungs ♀ 1805
Leukemia ♂ 3553
STRAIN-90
Uncertain ♀
Uncertain ♂ 842
Carc M Gl ♀ 758
Pul Inf ♂ 809
G3
G2
Carc Lungs ♀ 4352
Sarc M Gl Testicle ♂ 3117
Carc M Gl ♀ 449
Abs Rectum Sem Ves ♂ 508
STRAIN-85 G PARENT
STRAIN-450 G PARENT
Carc M Gl Prim Carc Lung ♀ 5924
Hypert Heart Int Inf ♂ 5183
G1
G1
Int Inf ♂ 6313
2 Carc M Gl Metas Lungs ♀ 7025
3 Carc M Gl ♀ 7247
Wounds ♂ 6984
Pseudo-Leuk ♂ 7585
Carc M Gl Metas Lungs & Diaphragm ♀ 8041
Adenoma Kidney ♂ 9907
Carc M Gl Metas Lungs ♀ 6675
G2
Carc M Gl ♀ 10734

CHART 8


STRAIN 338.
BRANCH III
G PARENT
G1
G2
G3
G4
G5
2 CARC M GL CARC PELVIS 5417 METAS LUNG
PAPILLOMA LUNG ARTERIAL SCLEROSIS 7736
INTESTINAL INFECTION 7899
INTESTINAL INFECTION 6851
ACCIDENT 6039
PUL INF 7210
LABOR 6967
CHRONIC NEPH 10504
SUBCUT SARC BACK 10262
WOUNDS 6033
CARC LUNG MULT LUNG METAS 10584.
3 CARC M GL 11868
CHRON NEPH 8329
WOUNDS 9182
PUL INF 7904
WOUNDS 8098
PUL INF 8397
WOUNDS 9575
2 CARC M GL MESOTHELIOMA OVARY 12552
CARC LUNG 9783
PUL INF 12024
CARC M GL 13921
2 CARC M GL HEM OVARY 10934
CARC M GL MULT LUNG METAS 14656
CARC M GL. 11681
RUPTURED AORTA 14715
CARC M GL MULT METAS LUNGS 12579
GENL SARCOMATOSIS SUBCUT AND BODYWALL 14777
HYDRONEPHROSIS 13090
2 CARC M GL 13896

G1 PARENTS OF CANCEROUS ANCESTRY BOTH DIED OF INTESTINAL INFECTION
THE RESULTING STRAIN SHOWS OVER 45% OF CANCER
0% INTESTINAL INFECTION

CHART 9

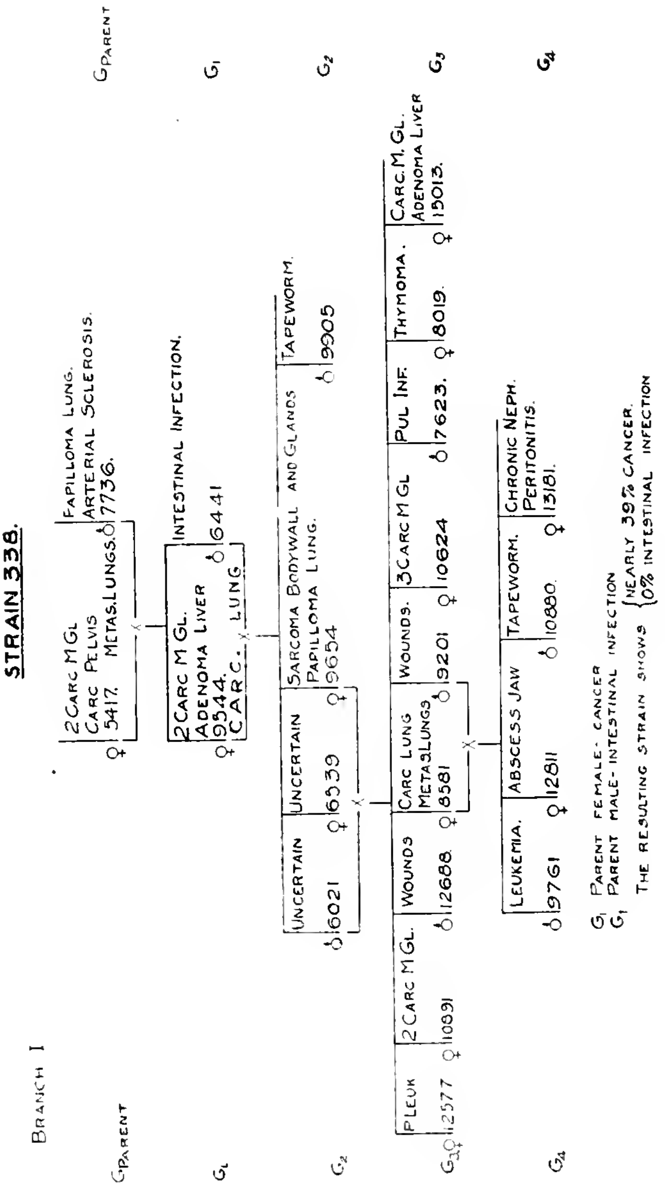

STRAIN 338.
Branch I
G Parent
G1
G2
G3
G4
2 Carc M Gl
Carc Pelvis
♀ 5417 Metas. Lungs.
Papilloma Lung.
Arterial Sclerosis.
♂ 7736.
2 Carc M Gl.
Adenoma Liver
♀ 9544.
Carc. Lung
Intestinal Infection.
♂ 6441
Uncertain
♂ 6021
Uncertain
♀ 6539
Sarcoma Bodywall and Glands
Papilloma Lung.
♀ 9654
Tapeworm.
♂ 9905
Pleur
♀ 12577
2 Carc M Gl.
♀ 10891
Wounds
♂ 12688
Carc Lung
Metas. Lungs
♀ 8581
Wounds.
♂ 9201
3 Carc M Gl
♀ 10624
Pul Inf.
♂ 7623.
Thymoma.
♀ 8019.
Carc. M. Gl.
Adenoma Liver
♀ 15013.
Leukemia.
♂ 9761
Abscess Jaw
♀ 12811
Tapeworm.
♂ 10880.
Chronic Neph.
Peritonitis.
♀ 13181.
G1 Parent female - cancer
G1 Parent male - intestinal infection
The resulting strain shows {nearly 39% cancer / 0% intestinal infection

Chart 10

of the liver, and carcinoma of the lung, exactly the locations and types of tumors represented in the female. Yet not one case of intestinal infection occurred in the family, although this family is bred out in four generations.

CHART 11

Strain 338. Branch XI

Here again in the first filial generation the individuals which head this branch showed on the one side carcinoma and on the other side intestinal infection, viz., female 6993 and male 6851. The two types of disease had, therefore, an equal chance of transmission if both were inheritable. Cancer, however, occurs in over 42 per cent, whereas not one case of intestinal infection ever appeared; and this in spite of the paucity of mouse cancers in general, and the overwhelming frequency of intestinal infections in mice.

Note the tendency in this branch to the occurrence of multiple primary tumors of the mammary gland: two in female 5417; four in female 6993; five in female 11307; seven in female 9929; two in female 12262. Note the close approximation in female 9921, filial generation 3, to the tumor production of her great grandmother, female 5417, parent generation of the branch. And in sharp contrast to this note the lone case of intestinal infection in male 6851, which left no imprint upon the strain.

CHART 12

Strain 338. Branch XIII

Again, in this branch of the family, both parents of the second filial generation died of intestinal infection, viz., female 6774 and male 7471. No instance of this disease ever appeared in the resulting strain. The female of the parent generation and the female of the first filial generation, however, carried carcinoma of the mammary gland and the male of the parent generation carried papilloma of the lung. Note the recurrence of these locations and types of tumors in the resulting strain, which showed over 41 per cent cancer, 0 per cent intestinal infection.

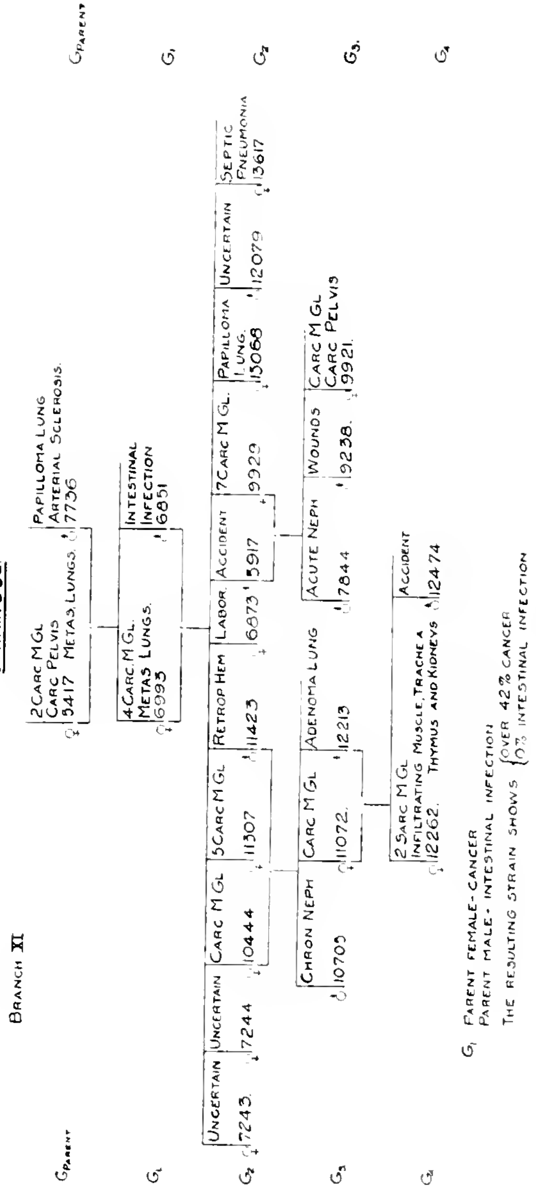
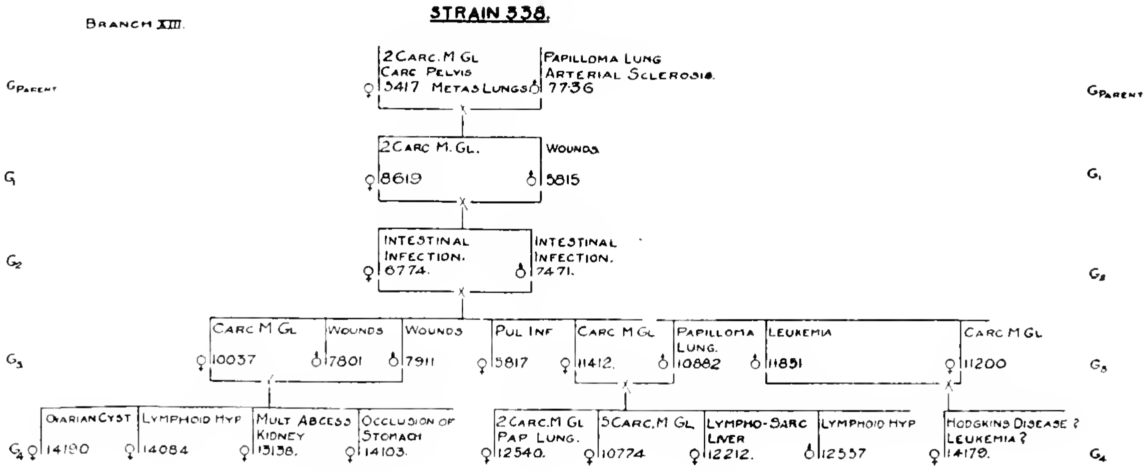

STRAIN 538
BRANCH XI
G PARENT
G1
G2
G3
G4
2 CARC M GL
CARC PELVIS
5417 METAS. LUNGS
PAPILLOMA LUNG
ARTERIAL SCLEROSIS.
7736
4 CARC M GL.
METAS LUNGS.
6993
INTESTINAL
INFECTION
6851
UNCERTAIN
7243.
UNCERTAIN
7244
CARC M GL
10444
5 CARC M GL
11307
RETROP HEM
11423
LABOR.
6873
ACCIDENT
5917
7 CARC M GL.
9929
PAPILLOMA
LUNG
13068
UNCERTAIN
12079
SEPTIC
PNEUMONIA
13617
CHRON NEPH
10705
CARC M GL
11072.
ADENOMA LUNG
12213
ACUTE NEPH
7844
WOUNDS
9238.
CARC M GL
CARC PELVIS
9921.
2 CARC M GL
INFILTRATING MUSCLE, TRACHEA
THYMUS AND KIDNEYS
12262.
ACCIDENT
12474
G1 PARENT FEMALE - CANCER
PARENT MALE - INTESTINAL INFECTION
THE RESULTING STRAIN SHOWS {OVER 42% CANCER
{0% INTESTINAL INFECTION

CHART 11


STRAIN 338.
BRANCH XIII.
G Parent
2 Carc. M Gl
Carc Pelvis
5417 Metas Lungs
Papilloma Lung
Arterial Sclerosis.
7736
G1
2 Carc M. Gl.
8619
Wounds
5815
G2
Intestinal
Infection.
6774.
Intestinal
Infection.
7471.
G3
Carc M Gl
10037
Wounds
7801
Wounds
7911
Pul Inf
5817
Carc M Gl
11412.
Papilloma
Lung.
10882
Leukemia
11851
Carc M Gl
11200
G4
Ovarian Cyst
14190
Lymphoid Hyp
14084
Mult Abcess
Kidney
13138.
Occlusion of
Stomach
14103.
2 Carc. M Gl
Pap Lung.
12540.
5 Carc. M Gl
10774
Lympho-Sarc
Liver
12212.
Lymphoid Hyp
12557
Hodgkins Disease ?
Leukemia ?
14179.

G_2 Parents of cancerous ancestry, both died of intestinal infection

The resulting strain shows {over 41% cancer
0% intestinal infection.

CHART 12

CHART 13

Strain 413. Branch A

In the first filial generation of this branch of the family the parent female, 7701, had carcinoma, the parent male, 6606, died of pulmonary infection. The branch carried over 33 per cent of tumors; 0 per cent of pulmonary infection.

CHART 14

Strain 413. Branch B

In branch B of this strain the introduction of intestinal infection by the first filial generation, male 6900, and the corresponding introduction of carcinoma by his mate, female 6874, produced the following results: cancer, over 22 per cent; intestinal infection, 0 per cent.

Note that in the second filial generation the parent male, 6998, died of pulmonary infection. There was no recurrence of pulmonary infection in his descendants.

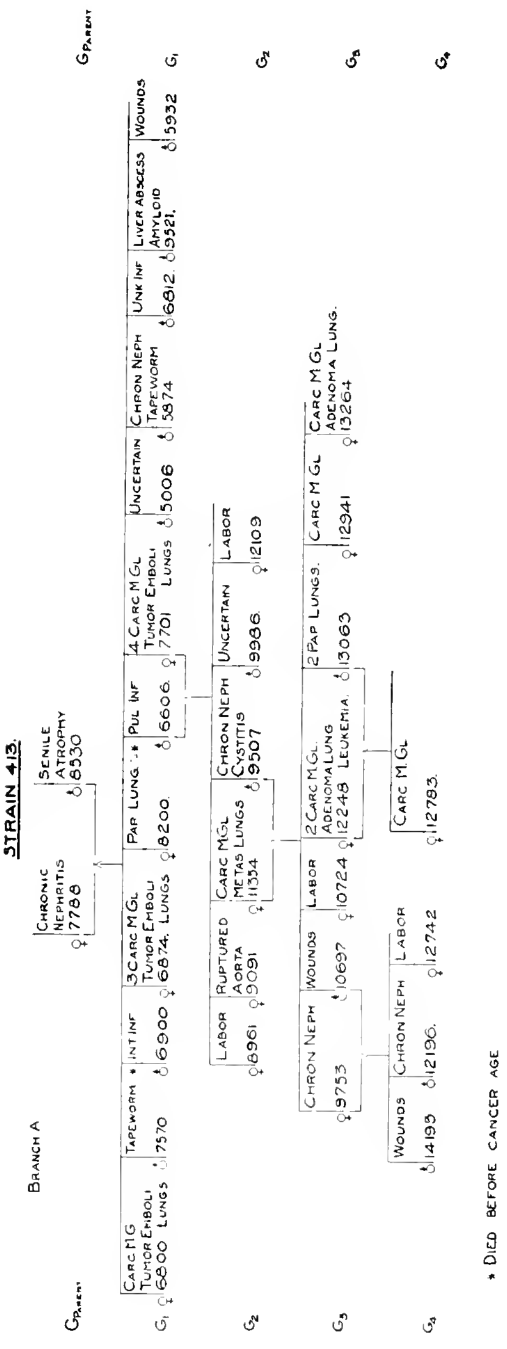
Note that the introduction of sarcosporidiosis by male 14835 (second filial generation) left no imprint upon the resulting strain. This male contracted sarcosporidiosis from a female with which he was hybridized long after his isolation from the family represented in this chart.

There was shown no inherited tendency to sarcosporidiosis; no inherited tendency to pulmonary infection; no inherited tendency 'to intestinal infections in this strain, although all these types of disease were represented in the parentage. On the other hand, the cancer tendency appears in over 22 per cent in the resulting strain.

CHART 15

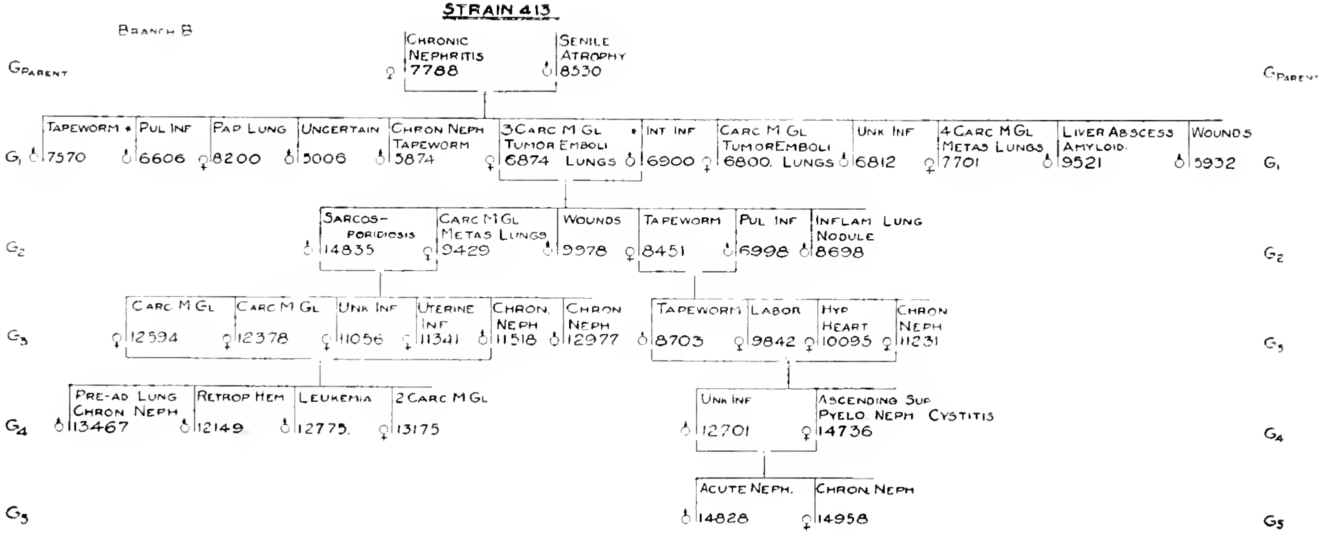
Strain 146. Branch C

Charts 15, 16 and 17 represent three lines in which branch I of strain 146 was bred out. Note here the conspicuous evidence of the segregating out of the cancer tendency and the non-cancer tendency as inheritable characters. In branch C, the


STRAIN 413.
BRANCH A
G PARENT
G1
G2
G3
G4
CHRONIC NEPHRITIS ♀ 7788
SENILE ATROPHY ♂ 8530
CARC M G TUMOR EMBOLI LUNGS ♀ 6800
TAPEWORM * ♂ 7570
INT INF ♂ 6900
3 CARC M GL TUMOR EMBOLI LUNGS ♀ 6874
PAP LUNG * ♀ 8200
PUL INF ♂ 6606
4 CARC M GL TUMOR EMBOLI LUNGS ♀ 7701
UNCERTAIN ♂ 5006
CHRON NEPH TAPEWORM ♂ 5874
UNK INF ♂ 6812
LIVER ABSCESS AMYLOID ♂ 9521
WOUNDS ♂ 5932
LABOR ♀ 8961
RUPTURED AORTA ♀ 9091
CARC M GL METAS LUNGS ♀ 11354
CHRON NEPH CYSTITIS ♂ 9507
UNCERTAIN ♂ 9986
LABOR ♀ 12109
CHRON NEPH ♀ 9753
WOUNDS ♂ 10697
LABOR ♀ 10724
2 CARC M GL ADENOMA LUNG LEUKEMIA ♀ 12248
2 PAP LUNGS ♂ 13063
CARC M GL ♀ 12941
CARC M GL ADENOMA LUNG ♀ 13264
WOUNDS ♂ 14193
CHRON NEPH ♂ 12196
LABOR ♀ 12742
CARC M GL ♀ 12783

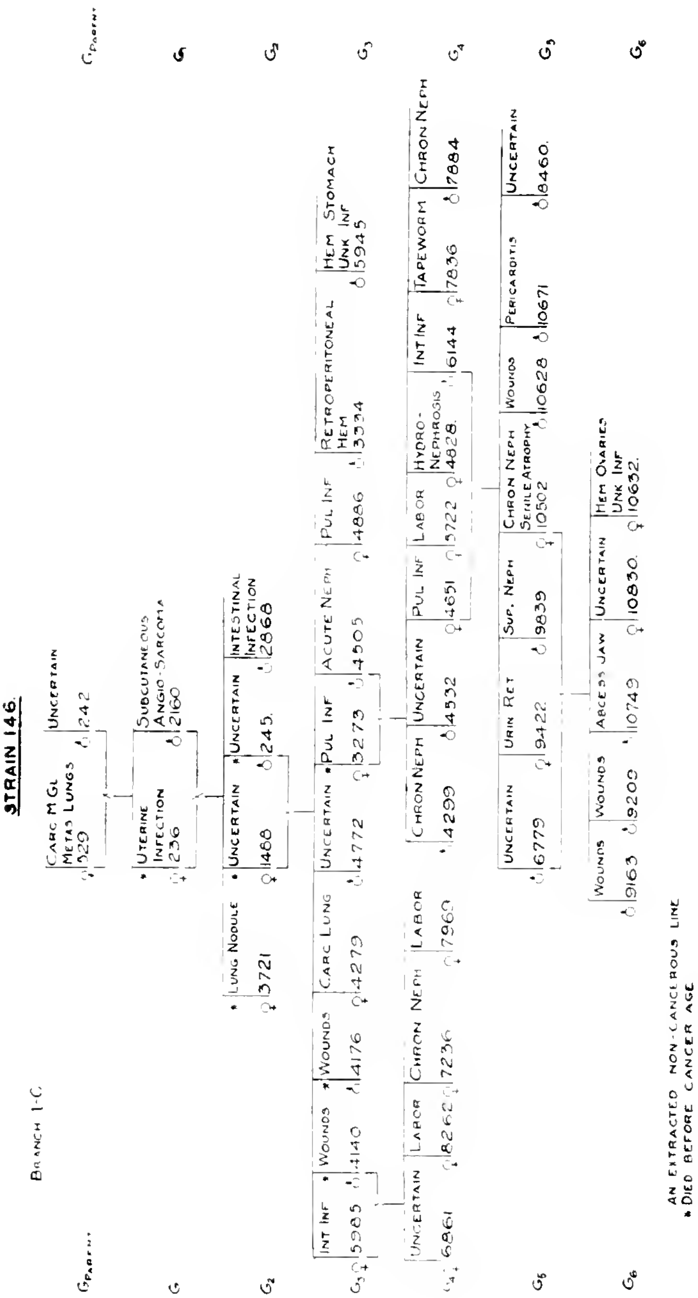
* DIED BEFORE CANCER AGE

CHART 13


STRAIN 413
BRANCH B
GPARENT
Chronic Nephritis ♀ 7788
Senile Atrophy ♂ 8530
G1
Tapeworm * ♂ 7570
Pul Inf ♂ 6606
Pap Lung ♀ 8200
Uncertain ♂ 5006
Chron Neph Tapeworm ♂ 5874
3 Carc M Gl Tumor Emboli Lungs ♀ 6874
* Int Inf ♂ 6900
Carc M Gl Tumor Emboli Lungs ♀ 6800.
Unk Inf ♂ 6812
4 Carc M Gl Metas Lungs ♀ 7701
Liver Abscess Amyloid. ♂ 9521
Wounds ♂ 5932
G2
Sarcosporidiosis ♂ 14835
Carc M Gl Metas Lungs ♀ 9429
Wounds ♂ 9978
Tapeworm ♀ 8451
Pul Inf ♂ 6998
Inflam Lung Nodule ♂ 8698
G3
Carc M Gl ♀ 12594
Carc M Gl ♀ 12378
Unk Inf ♀ 11056
Uterine Inf ♀ 11341
Chron. Neph ♂ 11518
Chron Neph ♂ 12977
Tapeworm ♂ 8703
Labor ♀ 9842
Hyp Heart ♀ 10095
Chron Neph ♀ 11231
G4
Pre-ad Lung Chron Neph ♂ 13467
Retrop Hem ♂ 12149
Leukemia ♂ 12775.
2 Carc M Gl ♀ 13175
Unk Inf ♂ 12701
Ascending Sup Pyelo Neph Cystitis ♀ 14736
G5
Acute Neph. ♂ 14828
Chron Neph ♀ 14958

* Died before cancer age

Chart 14


STRAIN 146
BRANCH 1-C
G PARENT
G1
G2
G3
G4
G5
G6
CARC M GL METAS LUNGS 229
UNCERTAIN 242
UTERINE INFECTION 236
SUBCUTANEOUS ANGIO-SARCOMA 2160
LUNG NODULE 3721
UNCERTAIN 1468
UNCERTAIN 245
INTESTINAL INFECTION 2868
INT INF 5985
WOUNDS 4140
WOUNDS 4176
CARC LUNG 4279
UNCERTAIN 4772
PUL INF 3273
ACUTE NEPH 4505
PUL INF 4886
RETROPERITONEAL HEM 3334
HEM STOMACH UNK INF 5945
UNCERTAIN 6861
LABOR 8262
CHRON NEPH 7236
LABOR 7960
CHRON NEPH 4299
UNCERTAIN 4532
PUL INF 4651
LABOR 5722
HYDRO-NEPHROSIS 4828
INT INF 6144
TAPEWORM 7836
CHRON NEPH 7884
UNCERTAIN 6779
URIN RET 9422
SUP. NEPH 9839
CHRON NEPH SENILE ATROPHY 10502
WOUNDS 10628
PERICARDITIS 10671
UNCERTAIN 8460
WOUNDS 9163
WOUNDS 9209
ABCESS JAW 10749
UNCERTAIN 10830
HEM OVARIES UNK INF 10632

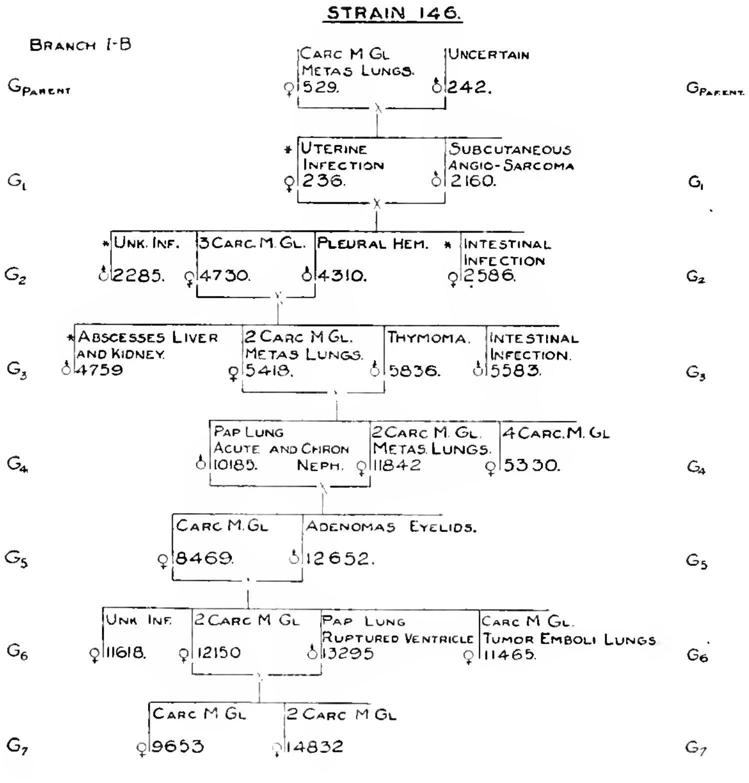
AN EXTRACTED NON-CANCEROUS LINE
* DIED BEFORE CANCER AGE

CHART 15

line in which it is bred out through female 3273 and male 4505 (second filial generation) is absolutely cancer free, an extracted non-cancerous line.

CHART 16

In branch B there arose an extracted cancerous line, every mouse of which that lived to more than barely six months of age, died of cancer.


STRAIN 146.
Branch I-B
G Parent
Carc M Gl Metas Lungs. ♀ 529.
Uncertain ♂ 242.
G Parent
G1
* Uterine Infection ♀ 236.
Subcutaneous Angio-Sarcoma ♂ 2160.
G1
G2
* Unk. Inf. ♂ 2285.
3 Carc. M. Gl. ♀ 4730.
Pleural Hem. ♂ 4310.
* Intestinal Infection ♀ 2586.
G2
G3
* Abscesses Liver and Kidney ♂ 4759
2 Carc M Gl. Metas Lungs. ♀ 5418.
Thymoma. ♂ 5836.
Intestinal Infection. ♂ 5583.
G3
G4
Pap Lung Acute and Chron Neph. ♂ 10185.
2 Carc M. Gl. Metas. Lungs. ♀ 11842
4 Carc. M. Gl ♀ 5330.
G4
G5
Carc M. Gl ♀ 8469.
Adenomas Eyelids. ♂ 12652.
G5
G6
Unk Inf. ♀ 11618.
2 Carc M Gl ♀ 12150
Pap Lung Ruptured Ventricle ♂ 13295
Carc M Gl. Tumor Emboli Lungs ♀ 11465.
G6
G7
Carc M Gl ♀ 9653
2 Carc M Gl ♀ 14832
G7

An extracted cancerous line
* Died before cancer age

Chart 16

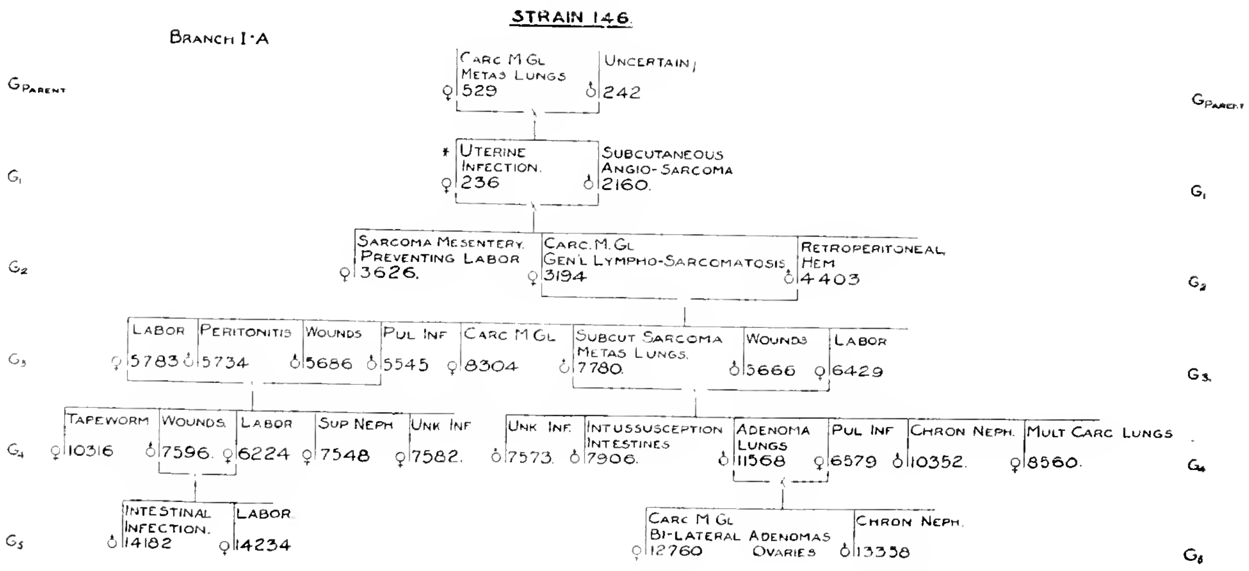
CHART 17

While in branch A, there arose a heterozygous line producing cancer and non-cancer in striking mendelian ratio [note also the three successive generations of sarcoma in filial generations 1, 2 and 3,] nothing remotely similar occurred with the infections represented in the strain. Throughout the three charts there is just one apparent transmission of pulmonary infection; and this is apparent only, since female 3273 was isolated with her sister, female 4886, and her daughter, female 4651, when she died of pulmonary infection. (Chart 15.) The other females in the cage caught the infection and died soon after, whereas none of her young isolated from her ever exhibited pulmonary infection. Further, the mating of female 4651, dying of pulmonary infection, with male 6144, dying of intestinal infection (both diseases being contracted after isolation from their young) failed to introduce either of these types of infection anywhere in the resulting strain, and this although it is almost impossible to keep any strain from being riddled with both these infections through contact contraction.

Note also that although the parent female of these three lines, viz., female 236, died of uterine infection, throughout all branches of the family resulting from this mating there was never another case of uterine infection. (Charts 15, 16 and 17.)

Throughout these charts, which are typical, the following facts stand out strikingly:

1. In every case where a cancerous individual is bred in, either in inbreeding or in hybridization, cancer comes out in a striking percentage in the resulting strain.
2. In no case where an individual dying of any type of well known infection is bred in, has there arisen a single case of the same infection in the resulting strain (except in one case of contact contraction) even where the families are bred out in many lines, in large numbers, and through many generations; and this although the infections tested are overwhelmingly prevalent among mice.


STRAIN 146.
Branch I·A
G Parent
G1
G2
G3
G4
G5
Carc M Gl Metas Lungs ♀ 529
Uncertain ♂ 242
* Uterine Infection. ♀ 236
Subcutaneous Angio-Sarcoma ♂ 2160.
Sarcoma Mesentery. Preventing Labor ♀ 3626.
Carc. M. Gl Gen'l Lympho-Sarcomatosis ♀ 3194
Retroperitoneal Hem ♂ 4403
Labor ♀ 5783
Peritonitis ♂ 5734
Wounds ♂ 5686
Pul Inf ♂ 5545
Carc M Gl ♀ 8304
Subcut Sarcoma Metas Lungs. ♂ 7780.
Wounds ♂ 5666
Labor ♀ 6429
Tapeworm ♀ 10316
Wounds. ♂ 7596.
Labor ♀ 6224
Sup Neph ♀ 7548
Unk Inf ♀ 7582.
Unk Inf. ♂ 7573.
Intussusception Intestines ♂ 7906.
Adenoma Lungs ♂ 11568
Pul Inf ♀ 6579
Chron Neph. ♂ 10352.
Mult Carc Lungs ♀ 8560.
Intestinal Infection. ♂ 14182
Labor ♀ 14234
Carc M Gl Bi-lateral Adenomas Ovaries ♀ 12760
Chron Neph. ♂ 13338

A heterozygous line in approximately Mendelian ratio

* Died before cancer age.

CHART 17

3. The cancer tendency and the non-cancer tendency segregate out conspicuously as inheritable characters which fulfill all the most exacting demands for a character which is inheritable, while there is absolutely no such segregation of any infection tendency. It is futile to *conjecture*[5] what might happen if one were dealing with "pulmonary infection" strains of mice, because one cannot get "pulmonary infection" strains of mice or any other infection strains of mice if one rules out the contact cause of transmission. On the other hand, the ruling out of contact as a cause of transmission of cancer does not modify in the least the per cent of cancer in the resulting strain.

4. From matings of mice both dying of cancer, it is possible to derive strains showing 100 per cent of cancer where the young can be kept free from infections long enough to live well into the cancer age.

5. From matings of mice where one parent dies of cancer it is possible to extract lines which follow mendelian ratios with striking precision, yielding extracted lines of cancerous, extracted lines of non-cancerous, and extracted heterozygous lines. These two facts develop consistently year after year in every test made and are not a matter of speculation. Whereas:

6. From matings of mice both dying of the same common infection (a) it is possible to derive strains which never show another case of this infection if the mice are properly protected from contact contraction, and (b) it is absolutely impossible to derive extracted strains of any common infection, if proper protection is afforded the mice from contact contraction.

7. From matings where one parent dies of a common infection, it is not possible to derive anything which approximates strains showing a segregating out of an infection tendency and a non-infection tendency, which is the hybridization test of inheritability.

8. Every mouse in the laboratory can be swept off by contact contraction of a virulent infection to which there has been exposure or, if an infected individual is properly isolated there need not

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[5] Erwin F. Smith, loc. cit.

be one other case of this infection throughout the laboratory, no matter how many offspring the individual has produced.

9. Whereas, contact is absolutely ineffective and has no bearing whatever upon the inevitable appearance of cancer in strains where this tendency has been bred in.

There is no similarity whatever between the inheritance behavior of cancer and the inheritance behavior of common infections.

PRELIMINARY NOTE ON THE POSSIBLE EFFECTS OF THE NERVOUS SYSTEM UPON THE GROWTH AND DEVELOPMENT OF TUMORS

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It is one of those strange phenomena which are occasionally met with in the history of medicine, that what one might think were very obvious anatomical facts or physiological relations, are for a long time either entirely overlooked, or do not receive the attention which they deserve. Thus it has for an indefinite time been accepted as a fact that genuine tumors, with the exception perhaps of those directly originating from nerves, as the neuromata, and of those more indirectly connected with them, as the multiple neurofibromata described by Recklinghausen, are not supplied with any nervous apparatus, with the exception perhaps of some vascular innervation, and even this has not been definitely ascertained for every kind of tumor. The literature on the subject is extremely scanty and consists mostly of a casual remark scattered here and there. William Cullen (1) in his "First lines of the practice of physic," 1789, looks upon cancer as caused by some disturbance of the nervous system. This, however, is not based on any anatomical investigation, but is simply in accordance with the teachings of the school to which he and his master Alexander Monro belonged, according to which a majority of human disease was supposed to be due to perverted nervous influence. Virchow (2) does not seem inclined to recognize any influence of the nerves upon the origin and growth of tumors. This opinion, however, seems to be based rather upon general considerations and not upon histological investigations, which, after all, the microscopic technique of his time would hardly have rendered feasible. John Marshall (3), in a very suggestive Morton lecture on "Cancer and cancerous diseases," urges the investigation of the relations of the nervous system to cancer as a very promising method of research. His own opinion is purely hypothetical and not based on any direct observation. To him, cancer is an "anarchical" growth of cells which perhaps have broken away from nerve control and thus can continue, stimulated probably by numerous other influences, in unlimited proliferation. He thus postulates a *biological* change in cancer cells by which they differ from the normal. At about the same time Klebs (4) remarks very briefly and without any further discussion, that the absence of nerves in tumors, especially malignant ones, may mean the diverting of normal inhibition from the tumor cells and thus may be made accountable for their unlimited proliferation. The great majority of the text books of pathology either make no mention at all of the question of the relation of nerves in tumors, or simply state that nerves in tumors have not been demonstrated. In 1897 Young (5), using the methylene blue supravital stain, made a rather extensive search for nerves in various kinds of neoplasms. He found nerve fibers, in some cases rather more plentifully than he expected, in about half of the cases he examined. He does not arrive at very positive conclusions, but is strongly inclined to the opinion that in several of his cases the nerves that he found were actual integral parts of the tumor. We are unable to consider this opinion as satisfactorily proven, especially as he does not show any constant relation of the nerve fibrils to the tumor cells, or to their possible functional activity. Since Young's paper we have not been able to find in the literature any other special research directed toward this problem. Borst (6) in his well known monograph reiterates the statement that nerves forming an integral part of genuine tumors have not been demonstrated, and points out the desirability of an exhaustive investigation of the subject. He says further, that it is quite obvious that in infiltrating tumors, nerves are very frequently found within the tumor tissue; that these, however, do not belong to the tumor itself, but are preexisting formations which are simply included into the tumor in the course of its encroachment upon the surrounding tissues.

A further study of this problem has been in the mind of one of us for a long time, and many years ago a number of experiments were made, which, however, could not, owing to the state of our knowledge at that time, produce any results. We have now concluded to take up the subject again.

It seems to us that there are two methods available for attacking the problem. One method would consist in an anatomical, especially histological, study, as complete as possible, of the actual relations of the nervous system to neoplasms; and if it should turn out, as seems probable, that tumors have no nerves of their own, an attempt to discover the biological conditions which determine this lack of innervation. The other method would consist in taking some transplantable tumor, inoculating it into various organs whose connection with the nervous system has been completely severed, and noting if there are any recognizable differences in the growth and development of the tumors in such organs, as compared with the result of inoculations into similar organs whose innervation has remained intact. Like everything connected with the great cancer problem in its totality, this special investigation is by no means an easy one, and almost insurmountable obstacles seem to block the road to definite conclusions. Nevertheless it has seemed to us worth while to devote our energies to this in the hope of perhaps attaining some, though possibly only a minimal, result.

The following set of experiments of which we present here a very brief report, were done in the first half of 1916 and extended over a series of fifty white male rats. The tumor employed was the well known Flexner-Jobling carcinoma. The organ selected for inoculation was the testicle. It is very easy to get at this organ and bring it outside of the scrotum and thus deprive it of all possible little nerve fibrils which might perhaps enter the testicle from its environment. As is well known, the main nerve supply of the testicle runs along the vas deferens and the spermatic vessels. There is no difficulty in stripping this funiculus of everything but the bare vessels. Unfortunately it was not possible to do this without interfering to some extent with the blood supply on account of the smallness and extreme fragility of the vessels. As a consequence, in practically every case in which the testicle was stripped of its nerves, blood vessels were also more or less damaged and a certain amount of atrophy of the glandular elements was pretty sure to follow. Of our fifty rats, six were denervated but not inoculated with tumor, because we wished to study the effect of the operation without any complicating circumstances. This, as stated before, resulted in a varying degree of atrophy of the glandular tissue, but we never encountered necrosis. Nine animals, including controls, denervated, and inoculated animals in about equal numbers, were found dead, and either so completely decomposed or eaten by their fellows, that nothing could be said about the result of the experiment, and they must therefore be excluded from the series. There remained then fourteen rats that had been inoculated in a testicle stripped of all its nerves. *All these fourteen rats developed tumors,* mostly exceptionally large. Of the twenty controls, that is to say, animals whose testicles were simply inoculated without interference with the nerve supply, either through the skin or more often through a very small incision into the skin, *seventeen showed no signs of tumor whatsoever, but were perfectly normal.* In one, the testicle was free from tumor, but there was some neoplasm in the omentum and sigmoid. It is doubtful whether this had any connection with the inoculation; at any rate, as the testicle was normal, it must count as negative in our series. In another rat there was a very small whitish-gray patch to be observed in the testicle, which however, under the microscope, did not show any signs of tumor formation, but was probably of inflammatory origin, and was distinguished by a great number of plasma cells. One other control showed an extremely small tumor. We can therefore state with confidence that *all* the rats that had been inoculated into testicles stripped of their nerves, very promptly developed exceptionally large and rapidly growing tumors, but that none of the controls, inoculated at the same time under the same conditions and with the same tumor, the testicle having its normal nerve supply, developed any tumor, with perhaps one single and insignificant exception. This result is the more striking when it is remembered that all investigators agree that the testicle is not a very favorable site for tumor transplantation; Woglom (7), indeed, has shown that the Flexner-Jobling tumor of the rat is transplantable into the testis of the animal, though he also finds that the resulting growths do not often attain the usual size of subcutaneous tumors.

We wish to mention just incidentally that we also tried a number of experiments with the object of testing the effect of Scharlach R. upon denervated testicles. Concentrated solutions of the dye in olive oil were injected into the testicle, sometimes alone, sometimes with aleuronat in order to produce some mechanical irritation. The experiments led to no result. Only very insignificant quantities of the Scharlach could be injected into so small an organ and this it seems was rapidly carried away by the lymphatics. We obtained some very beautiful specimens of Scharlach injections through the great lymph trunks of the abdomen and into the centrum tendineum, and even into the thoracic cavity. What ultimately became of it we did not investigate.

Returning to our tumor inoculations, we realize that no very positive conclusions can be derived from them. The series is altogether too small, and must be extended over a considerably greater number of animals; other organs must be tested in the same way, and above all there is one obvious objection that must be overcome. A method must be found by which an organ can be completely severed from all its connections with the nervous system without at the same time interfering in the least with its blood supply, for it may well be conceived that the general atrophy following the operation on the rat's testicle lowers the normal tension and mutual balance of the tissues to such an extent as to give the implanted foreign tumor element more room and a better chance for development. That such a mechanism is possible or even probable has, as far as we can see, not yet been proven, but nevertheless the possibility must be taken into account. We offer this series of experiments, therefore, merely as a preliminary notice and refrain from drawing any conclusions, desiring merely to place on record results which, at least, are somewhat encouraging.

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TUMOR IMMUNITY IN THE CHICK EMBRYO

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The biological study of transplantable tumors in animals has developed and established certain principles, some of which have later been found to have interesting variations; thus, it has been shown that a tumor from one species will not grow progressively in another species of animal. But there are exceptions to this general rule in the fact that the young of one species are susceptible, for a certain period during their development and growth, to tumors from another species. This has been shown to be true when rat and mouse tumors are inoculated into the chick embryo (Murphy (1), Stevenson (2)), and also when mouse tumors are inoculated into new-born rats (Bullock (3)). In a later communication Murphy reported that this period of susceptibility could be terminated in the chick embryo, by the introduction of a graft of adult chicken spleen. On this observation and the work of Da Fano (7) he based his ingenious hypothesis regarding the function of the lymphocyte in immunity to transplanted tumors. This hypothesis was weakened, however, by Bullock's (3) discovery that new-born rats, ordinarily susceptible to inoculation with a mouse tumor, did not become refractory even when adult rat spleen and tumor were simultaneously inoculated. This is in exact contradiction to the findings of Murphy in the chick embryo; in each case the attempt is made to immunize against a tumor of a certain species growing upon a foreign soil, with tissues derived from this foreign soil.

This interesting statement of Murphy's, according to which the chick embryo presents an exception to the findings in another species of animal that has a similar brief period of susceptibility, seemed to indicate that a further investigation of the immune reaction of the chick embryo to tumors of the rat and mouse was necessary. With this in view, a short series of experiments was undertaken to discover whether or not tumors other than the Jensen rat sarcoma, which Murphy (4) used in his experiments, react in a similar manner.

EXPERIMENTS

The eggs used in the experiments were derived from one source. The technique employed in the inoculations, which was similar to that used by Murphy (1), was as follows: The location of the embryo having been determined by candling, the shell was cut through at a point over the embryo with a sharp knife, and a small square of shell removed. The vitelline membrane was then torn through and the tumor and spleen grafts placed through this opening upon the allantois with a curved forceps. The piece o shell was carefully replaced and sealed with paraffin. Aseptic precautions were observed throughout the procedure.

Murphy having stated (4) that adult chicken spleen was the tissue most potent in inhibiting the growth of tumors in the chick embryo, this tissue was used in amounts of 0.005 gram. Since Murphy (4, 5) neglected to state the amount of spleen used, it was necessary to determine this arbitrarily, judging from the quantity necessary to produce immunity in the mouse. Microscopic examination of sections of the spleen used was always made, to be sure that they were normal. The eggs were inoculated on the seventh day of incubation and the grafts removed on the seventeenth or eighteenth day of incubation. The inoculations of the spleen and tumor were made simultaneously, 0.003 gram of tumor tissue being used.[1] The grafts were separated on the membrane about 1 cm. before the egg was sealed. A series of control eggs was inoculated with tumor alone at the same time that tumor and spleen were used.

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[1] In previous publications from the Imperial Cancer Research Fund and from this laboratory the inoculation dose, when the needle method is used, has been estimated as 0.01 or 0.02 gm.; but such grafts have recently been found, as a matter of fact, to weigh about 0.002 and 0.003 gm. respectively.

According to the above procedure, a mass of the Ehrlich mouse sarcoma was cut up into bits of 0.003 gram and inoculated into a series of embryos. Adult chicken spleen was inoculated at the same time. Ten days later the eggs were opened, the site of inoculation removed and serial sections cut through any areas of thickening that were present. This was done because it was realized that while such areas are plainly visible in gross, it is practically impossible to tell, even with a hand lens, whether growing tumor is present or not. Microscopic examination of the sections showed that one of these thickened areas contained a large piece of healthy splenic tissue as well as a fragment of tumor in a healthy growing condition. The tumor cells were similar to those seen in control grafts (without spleen) on the chick membranes, showing many mitotic figures which indicated undoubted growth activity. The connective tissue reaction about the tumor was perhaps a little more dense than is usually seen in the controls without spleen, and the wandering-cell reaction was more abundant than around the tumor in the controls. This modification of the reaction on the part of the chick tissues from that seen around control tumors had not, however, influenced the growth of the tumor.

The outcome of this experiment was so unexpected that another series similar to the above was inoculated with the same tumor, the Ehrlich mouse sarcoma. This gave several grafts which presented in the same section not only healthy spleen on the membranes of the chick, but also large healthy growing tumor grafts. In some sections the splenic graft was found to be in close proximity to the tumor, but in this position it seemed to have as little influence on the growth of the tumor tissue as splenic grafts at a greater distance. The wandering-cell reaction around the tumor was undoubtedly increased; it was composed not of lymphocytes, however, but almost entirely of cells belonging to the myeloid, or granular leucocyte, series.

Upon careful consideration of these findings, it was thought that the reason for this variation from the results reported by Murphy (4) must be due to the fact that the Ehrlich mouse sarcoma is a tumor against which no immunity can be created in the mouse, and that, consequently, immunity could not be induced in the chick. As immunity is very easily induced in the rat against the Jensen rat sarcoma, which Murphy used, it appeared possible that tumors against which immunity can be obtained in the animal would not grow in the presence of spleen in the chick embryo, while tumors that cannot be immunized against in the animal will grow without hindrance in the chick also. With this in view, a study was made of the influence of spleen upon the growth of two types of tumor—those against which immunity can, and those against which it cannot, be obtained in the animal.

The tumors of the former group, against the implantation of which immunity can be produced by suitable methods, were the Crocker mouse carcinoma 11, the Imperial Cancer Research Fund mouse carcinoma 63, the Jensen rat sarcoma, and the Crocker rat sarcoma 8. To these were added the Crocker mouse sarcoma 180, against which immunity cannot be produced by any method now known. In these experiments the technique, the dosage, and the source of the eggs were similar to those in the previous series. In order to show that the tumors in the first group had not varied in their immunity reactions as previously determined in this laboratory, a portion of the same growth that was inoculated into the eggs was also implanted in animals. These animals had been previously immunized in the usual fashion with embryo skin. The failure of the tumors to grow proved that all were still susceptible to immunizing agents.

In the series of embryos into which mouse carcinoma 63 was inoculated, 40 per cent of the specimens contained both the splenic graft and the tumor graft in the same section, and microscopic study of the tumor in these sections showed it to be in a healthy growing condition in every case. The reaction on the part of the connective tissue was no more intense than that which occurred around the control grafts in the chick. The wandering-cell reaction, however, was more dense than in the control tumors in the chick, but the predominating cells were again of the myeloid series. The tumor cells contained quite as many mitotic figures as did those in the control tumors in the chick, and the size to which the tumors had grown in the presence of spleen was equal to the control tumors in the chick in every instance. The splenic graft was well organized and in a healthy condition.

In 47.6 per cent of the specimens recovered from the series into which mouse carcinoma 11 was inoculated with adult chicken spleen, tumor and spleen were found growing together on the membrane. Serial sections of these showed the spleen well organized and the tumor in a healthy condition with numerous mitotic figures in the nuclei. Careful study of these sections revealed nothing further than was noted in the series with the 63 tumor. The tumor showed active growth and was extending rapidly into the chick tissue. The reaction on the part of the chick was similar to that seen in sections of the above series, the wandering-cell reaction being here also much more abundant and composed of cells of the myeloid series.

The series in which the Jensen rat sarcoma was inoculated together with adult chicken spleen, proved of great interest, since this tumor was used in the experiments of Murphy (4), who stated that it would not grow under these conditions. The inoculations were made in a manner identical with those of the foregoing experiments and the results were equally successful. Of the series, 24.1 per cent of the grafts recovered after a residence of ten days in the chick showed on section that both tumor and spleen grafts were growing together on the membrane. The tumors contained many cells in the process of mitotic division. The connective tissue reaction around the tumor was no more than was found around the control tumors without spleen grafts. The wandering-cell reaction, however, was undoubtedly increased around the tumor, in comparison to the controls, but the cells comprising this reaction were again elements of the myeloid or granular series.

The rat sarcoma 8, which is of the large round cell type, did not appear to grow well in the chick, at least in this series. The control tumors were small and the tumor cells showed only an occasional mitotic figure. The tumor grafts recovered where the spleen and tumor were introduced at the same time, were likewise small and showed only an occasional mitotic figure. Only 6.6 per cent of the living embryos showed a double growth. The spleen graft was found to be in a healthy condition. The reaction on the part of the chick tissue showed some increase in the fibrous tissue reaction, and also an increase in the wandering-cell reaction, which, as in all previous instances, was composed of myeloid cells. Yet the grafts accompanied by spleen were no smaller than those growing alone, a most significant finding, for this tumor, which, in the present experiment at least, grew poorly in eggs, should have been the first to show any inhibitory action that might have been exerted by the spleen.

The mouse sarcoma 180 also grew when inoculated with adult chicken spleen. The control series with this tumor was of interest, as it gave a very low percentage of takes (18.1 per cent) and the wandering-cell reaction was rather intense. The tumor cells showed only an occasional mitotic figure. This tumor, growing in the presence of spleen in 13.8 per cent of living embryos, showed at times many mitotic figures and numerous giant cells, while in other grafts the tumor cells were more spindle-shaped and contained only an occasional division figure. Inspection of the records showing the outcome of routine inoculations of this growth into mice, proved it to be in a negative phase at the time it was used in this experiment. The phase appears also in the egg in the control series, but even this devitalized condition did not render the tumor susceptible to the influence of adult chicken spleen.

TUMORS WITH SPLEEN				CONTROL TUMORS WITHOUT SPLEEN		
Tumor	Living embryos	Tumor and spleen both growing	Percentage	Living embryos	Tumor growing	Percentage
11	21	10	47.6	8	6	75.0
E S	29	6	20.6	10	3	30.0
J R S	29	7	24.1	10	7	70.0
63	30	12	40.0	7	3	42.8
180	36	5	13.8	11	2	18.1
R S	15	1	6.6	8	4	50.0

The percentage of takes with the various tumors varied with each series. In some it almost equaled that of the controls, while in others it was smaller. It seemed that this variation might be explained by the mere mechanics of the operation of inoculation, for in some instances the graft will float up and stick to the shell. Therefore, where a double inoculation was made, the possibility of having both of the grafts grow would be less than if a single particle of tissue had been introduced. This supposition was corroborated by a series of 83 embryos in which double inoculations were made; that is, where two pieces of tumor were inoculated at the same time. The result of this experiment was that although 75 per cent of the living embryos had tumors, only 33 per cent of them showed double grafts; yet the tumor used had been carefully selected because it never produces concomitant immunity in the mouse. The lower percentage of success where two tumor grafts are simultaneously inoculated easily explains why, in some of the tumor-spleen eggs, the percentage of takes of the tumor was smaller than in the control series.

DISCUSSION AND SUMMARY

Many suggestive points are brought up by these observations. Probably the most interesting is their substantiation of the results obtained by Bullock (3), who reported no inhibition of the growth of mouse tumors in the new-born rat after the introduction of adult rat spleen.

Another finding of interest is that no effect is exerted by chicken spleen even upon tumors of low vitality, although one would expect them to succumb to a much more delicate influence. The dosage of spleen used was sufficient to induce a distinct immunity in the mouse, and, if the relative weights of the animals be taken into account, should have been more than sufficient in the chick.

The recent work of Danchakoff (6) has an important bearing upon the increased number of wandering-cells around the tumor grafts under the influence of spleen. Investigating the influence of splenic grafts on the allantois upon the hematopoietic system of the chick embryo, she made the observation that when spleen is planted on this membrane it stimulates the entire mesenchyme of the chick to the production of blood cells of various types. This myeloid hyperplasia is not confined to the mesenchyme tissue of the body of the chick, but includes that of the membranes as well. The increased cellular reaction around tumor grafts when spleen is present is most logically explained on a basis of the general myeloid hyperplasia which takes place under this influence. The increase undoubtedly is due to the general change in the chick under these circumstances, for according to the laws of immunity so far known, any effect that the spleen might have upon the tumor must come through a general reaction on the part of the chick, and not by a direct reaction between the spleen and tumor.

In contrast to Murphy's statement (1), we have seen that the reaction about a control tumor in the chick may be distinct; yet it is never so abundant as it is about those grafts that are accompanied by spleen. The fact that the reaction of the wandering-cells around the tumor grafts when spleen is present increases so much over that around the control grafts, as was noted by Murphy (4), is also of interest, in view of questions regarding the amount of spleen necessary to produce immunity, if, indeed, this can be produced at all. It seems probable that dosage has little or no bearing on the question of the splenic influence, provided the amount of spleen present be sufficient to induce the general reaction. Nevertheless, whether or not a greater reaction with possible inhibition of the tumor could be induced by very large doses of spleen, is a question that must be investigated. It is reasonable to suppose, however, that where this reaction had been induced in the chick and the wandering-cells been called out in great numbers, an increased amount of spleen would not be able to increase a reaction already so greatly magnified, in such manner as to have any effect on the tumor. The vitality of the chick is reduced by the spleen grafts, as Danchakoff (6) has shown, and this would make the determination of the spleen dosage a very difficult problem.

It was observed during the experiments above presented that the location of the splenic graft in relation to the growth had no influence on the condition of the tumor. It made no difference whether the spleen was in close proximity to the tumor, or whether it was at a considerable distance from it; indeed, some of the largest tumors obtained were in close proximity to the splenic grafts. A few were in actual contact with the spleen, and some of these had even invaded the splenic tissue.

CONCLUSIONS

It has been shown:

1. That all the tumors used grow without hindrance in the chick embryo in the presence of adult chicken spleen.
2. That wandering-cells are more abundant around the tumor, when spleen is present, but that these are mostly of the myeloid or granular leucocyte series, and not lymphocytes.
3. That even a tumor in its negative phase is not influenced by adult chicken spleen.
4. That the growth of a tumor in the chick in the presence of spleen has no relation to its general immune reactions in an animal of the same species as that from which the tumor was derived.

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PLATE 1

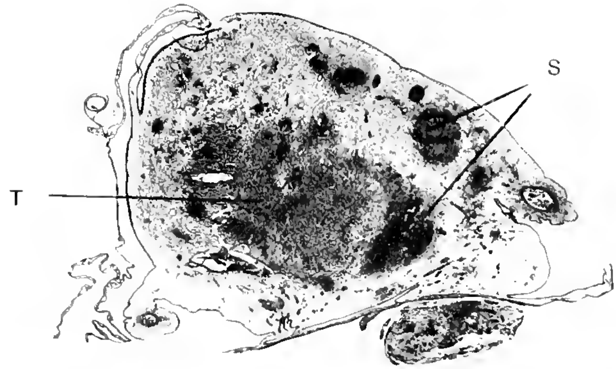
FIG. 1. Crocker mouse sarcoma 180 and graft of adult chicken spleen on the allantois. *T* is tumor, *S* is spleen. × 11.

FIG. 2. Tumor graft from figure 1. × 400.

FIG. 3. Control showing optimum growth of tumor 180 in the chick. × 400.

FIG. 4. Control for the series of tumor 180 and spleen. × 400.

FIG. 5. Splenic graft in figure 1. × 400.



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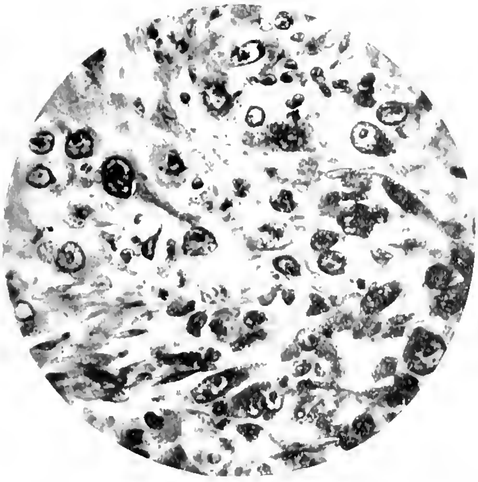

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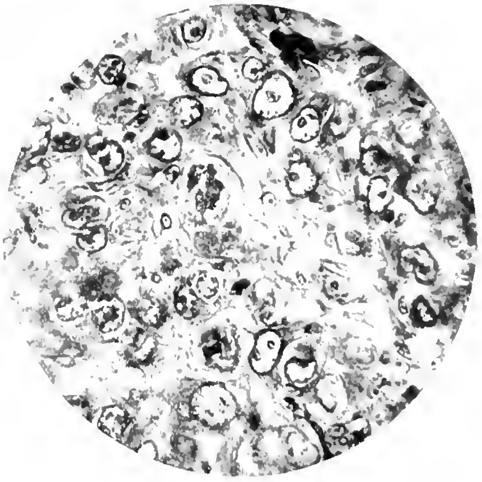

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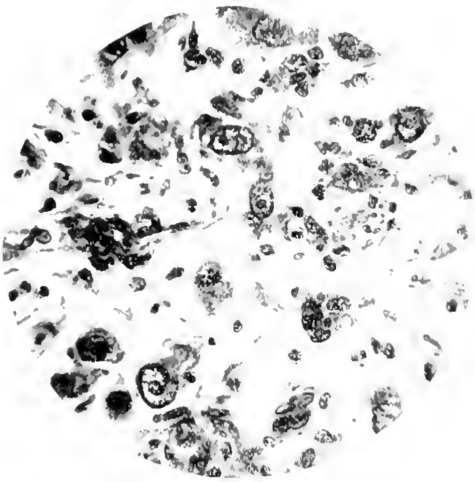

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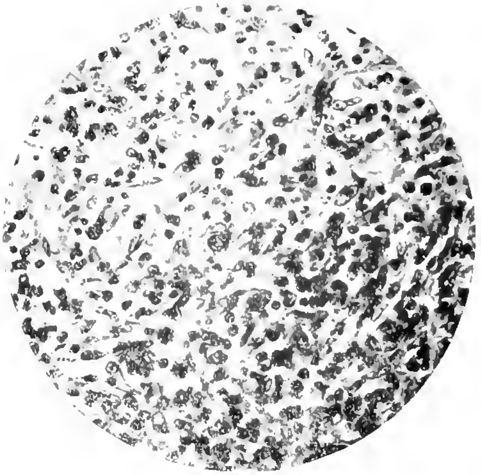

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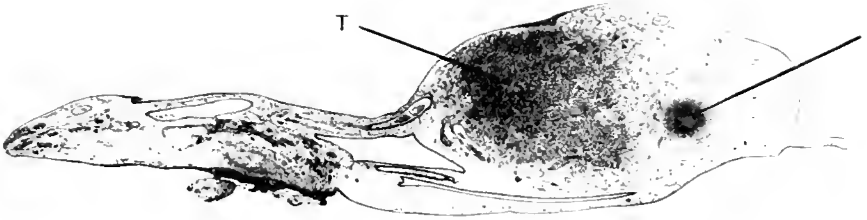
PLATE 2

FIG. 6. Ehrlich sarcoma and the spleen graft. *T* is tumor, *S* is spleen. × 11.

FIG. 7. Splenic graft from figure 6. × 400.

FIG. 8. Ehrlich sarcoma growing in the presence of spleen from figure 6. × 400.



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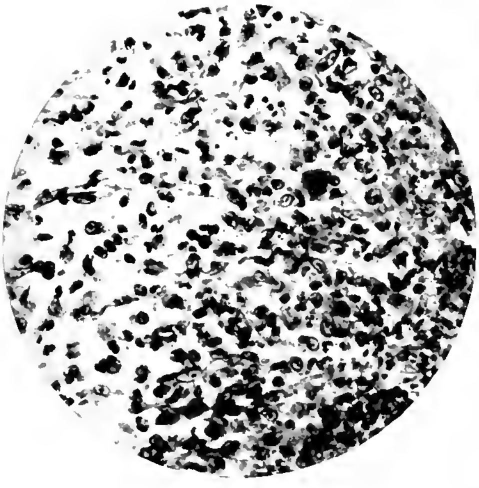

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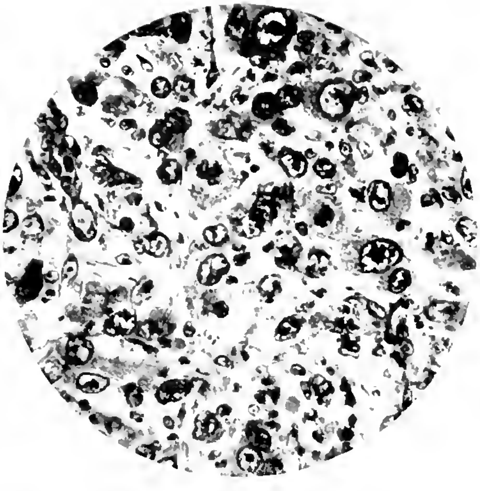

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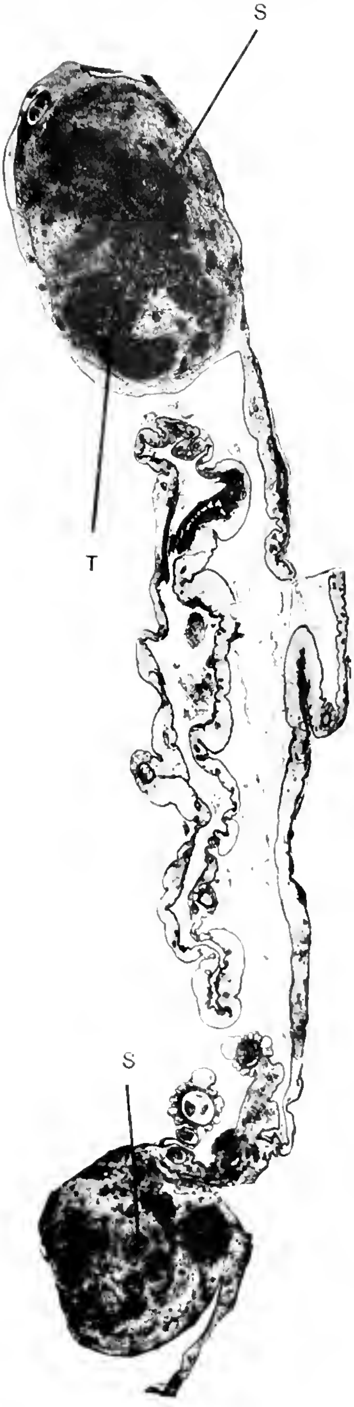
PLATE 3

FIG. 9. Low power showing the relation of Crocker mouse carcinoma 11 to the graft of adult chicken spleen. *T* is tumor, *S* is spleen. × 11.

FIG. 10. Tumor 11 from figure 9 showing its healthy growing condition and the reaction on the part of the wandering cells. × 100.

FIG. 11. Splenic graft from figure 9 showing its healthy condition. × 100.



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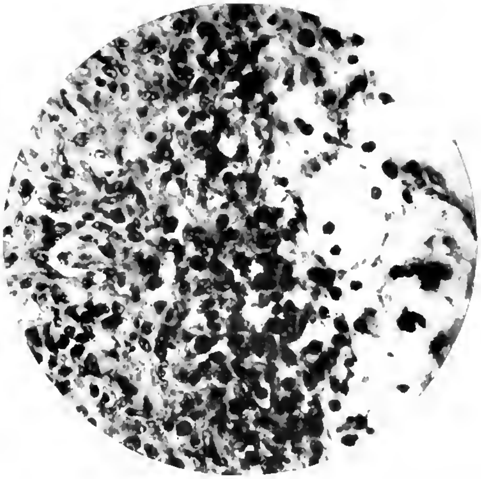

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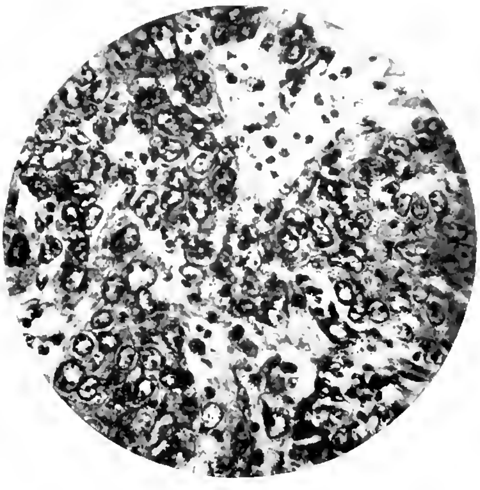

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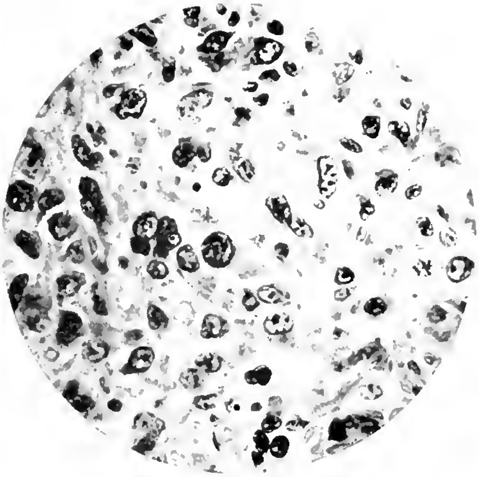
PLATE 4

FIG. 12. Graft of Crocker rat sarcoma S on the chick membranes. The tumor and spleen are seen together in the one nodule. *T* is tumor, *S* is spleen. × 11.

FIG. 13. The control tumor for the series with spleen. × 400.

FIG. 14. Tumor S from figure 12. × 400.

FIG. 15. Splenic graft from figure 12. × 400.



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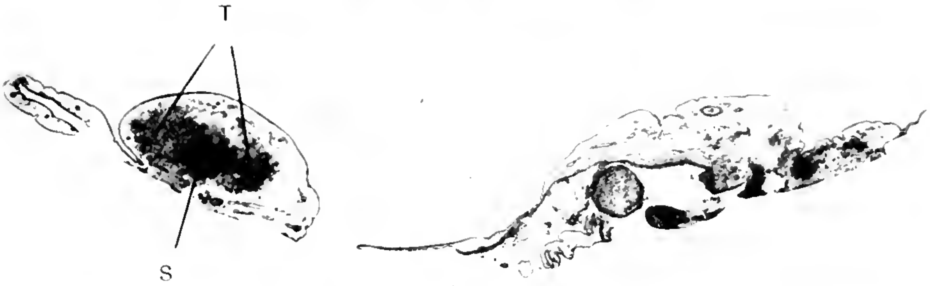

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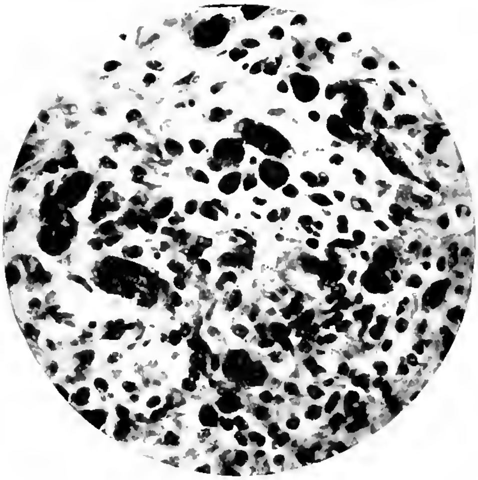

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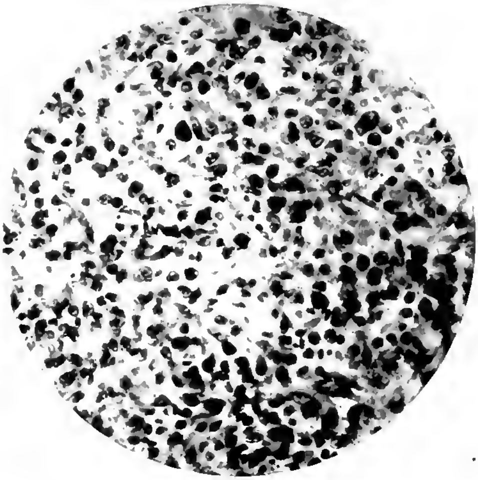

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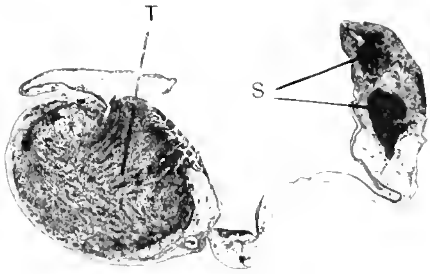
PLATE 5

FIG. 16. Jensen rat sarcoma growing in the presence of the graft of adult chicken spleen. *T* is tumor. *S* is spleen. × 11.

FIG. 17. Jensen rat sarcoma from figure 16. × 400.

FIG. 18. Splenic graft from figure 16. × 400.



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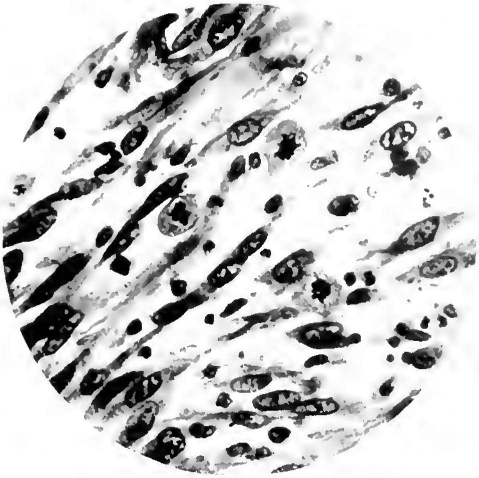

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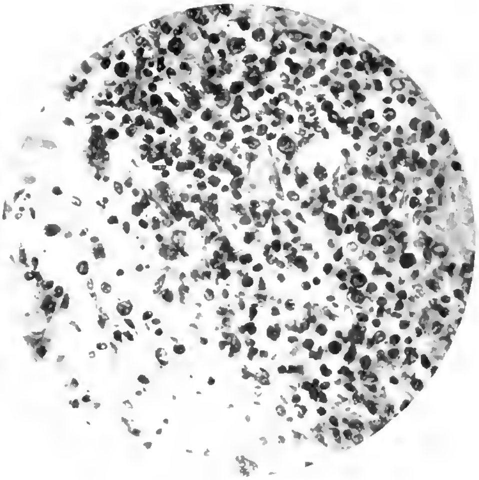

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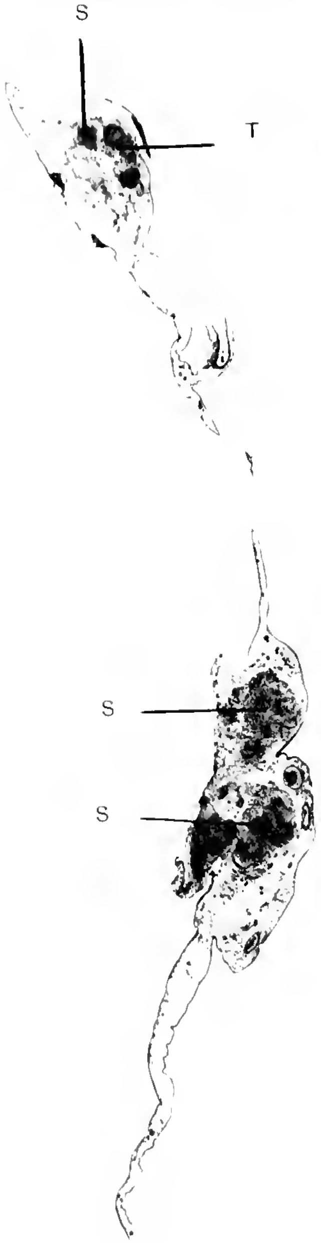
PLATE 6

FIG. 19. Imperial Cancer Research Fund mouse carcinoma 63 growing in the presence of spleen on the allantois. *T* is tumor, *S* is spleen. × 11.

FIG. 20. Tumor 63 from figure 19. × 400.

FIG. 21. Splenic graft from figure 19. × 400.



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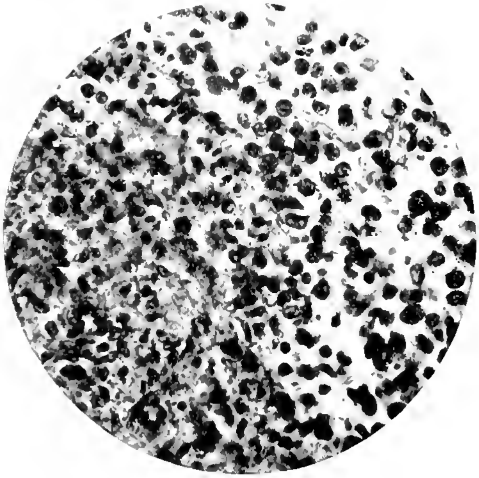

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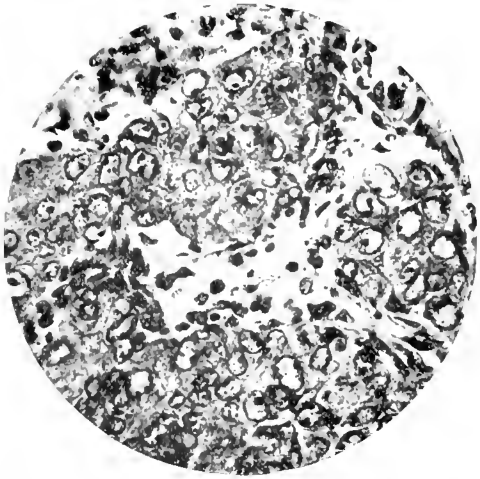

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ON THE ALLEGED INCREASE OF CANCER

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The question whether cancer or any other disease is becoming more prevalent is one upon which statistics can throw much light. Indeed, it must be answered by the help of statistics, f it is to be answered at all. At the start distinctions must be recognized between sickness and death, diseases and causes of death, morbidity and mortality. The prevalence of a disease is measured by the number of times the disease occurs in a population group in a unit of time, or the morbidity. Thus, the prevalence of typhoid fever as a disease is stated as the number of cases annually per 100,000 persons. The prevalence of a cause of death is measured by the number of times a death from that cause occurs in a population group in a unit of time, or the mortality. Thus, the prevalence of typhoid fever as a cause of death is stated as the number of deaths from typhoid fever annually per 100,000 persons. The prevalence of either a disease or a cause of death through at least two periods of time must be measured before the increase or decrease of either can be determined. The deadliness of a disease, that is, the number of deaths in each hundred or thousand cases, sometimes called the case mortality or case fatality, commonly differs in different regions and changes from one period to another. In consequence the morbidity from a given disease is not a satisfactory index of its mortality, and changes in the morbidity between two periods seldom, if ever, run exactly parallel with those in mortality.

Cancer is both a disease and a cause of death. The question whether the disease is increasing can be answered only by a well developed and trustworthy system of morbidity statistics. No country or city in the world now has such a system, because none has a good registration of sickness in the entire population.

Consequently, no significant statistical study of the increase of cancer as a disease, or of cancer morbidity, has yet been written or can be. The present paper is concerned only with the alleged increase of cancer as a cause of death. But before approaching that topic it should be conceded that, strong as the argument is for the increase of cancer mortality, the argument for the increase of cancer morbidity is probably stronger. In other words, the case fatality of cancer is probably less than formerly. This would follow from the increasing proportion of operations to cases, in some of which the disease never recurs, in others of which the progress of the disease is stayed, affording in the longer interim an increased chance for the action of new causes of death. The same conclusion is supported by other improvements in methods of treating cancer, all tending to prolong the patient's life and increase the chance of an intervening death from some other cause.

The question with which this paper is concerned may be phrased thus: Is cancer mortality increasing? In nearly all parts of the civilized world the *reported* mortality is increasing rapidly. This is the great outstanding fact clearly revealed by the figures. The opinion that the real mortality is increasing which now prevails among medical men and the general public rests in apparent security upon this broad statistical foundation. There is no denying that the figures make out a strong case for this conclusion, and that it cannot be rebutted by general statements about improvements in the diagnosis of cancer, or about the increasing proportion of the population consisting of elderly persons especially liable to cancer. Doubtless such considerations warrant the opinion that the true increase in the death rate from cancer is less than the increase shown by the figures, an opinion entertained, I believe, by all students of the subject, even those who seem to accept the published figures at their face value. But these general statements are far from adequate to justify the denial of any increase or the belief that an increase is improbable.

Studies in this field are of two main types, which may be distinguished as intensive and extensive. In the case of an intensive study the figures examined speak for only a limited area, usually a city, and are analyzed in various ways, in order to disentangle their true meaning and to confirm or correct one inference by another. In the case of an extensive study, the figures speak for many different districts, and reliance is placed upon the corroboration derived from similar indications in many or all.

Nearly all studies of the increase of cancer have been made by physicians who have done little work in statistics, but two careful examinations have been made by statists of high repute and are so much more thorough and significant than any of the others that they stand in a class by themselves. One, published by Messrs. King and Newsholme in 1893, is a model of an intensive investigation, mainly into evidence derived from the city of Frankfort in Germany.[1] The other, published by Mr. F. L. Hoffman in 1915, is the only example with which I am acquainted of an extensive investigation.[2] But two other international compilations of vital statistics, including statistics of cancer, have recently been published[3] and a large body of American material for the decade 1900–1909 and the year 1914 has been brought together.[4] All these contain uninterpreted figures which can be made to throw no little light upon the alleged increase of cancer mortality.

In view of this situation, the objects at which I aim are:

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[1] On the Alleged Increase of Cancer, in Royal Society, Proceedings, liv, pp. 209–242. Read May 4, 1893. To be cited henceforth in this article as King and Newsholme. Republished in Institute of Actuaries, Journal, xxxvi, part II (July, 1901), pp. 120–150.

[2] The Mortality from Cancer Throughout the World, The Prudential Press, Newark, N. J., 1915. To be cited henceforth in this article as Hoffman.

[3] Statistique Internationale du Mouvement de la Population, in two volumes, Paris, Imprimerie Nationale, 1907 and 1913. To be cited henceforth in this article as March. Statistique Démographique des Grandes Villes du Monde, 1880–1909, in two parts. (Communications statistiques, Bureau municipal de Statistique d'Amsterdam, nos. 33 and 40.) Amsterdam, Johannes Müller, 1911 and 1912. To be cited henceforth in this article as Falkenburg.

[4] United States Bureau of the Census, Mortality Statistics, 1909, Washington, 1912; and Mortality from Cancer and Other Malignant Tumors in the Registration Area of the United States, 1914, Washington, 1916.

1. To review the intensive study of King and Newsholme and to present new evidence from Frankfort for the period 1890–1913 by which their argument may be tested.
2. To summarize the results of other intensive studies which have been published since 1893.
3. To make a new extensive study by reviewing and interpreting the large body of evidence recently published in the international and American sources with no fuller interpretation than that found in Mr. Hoffman's volume.

Such a paper seems the more opportune for two reasons: first, because the two most significant studies reach diametrically opposite conclusions, King and Newsholme alleging that "the increase in cancer is only apparent and not real,"[5] and Hoffman maintaining "that the mortality from cancer is increasing at a more or less alarming rate throughout the entire civilized world;"[6] and secondly, because little, if any, attention has hitherto been paid to the light thrown on this problem by recent international compilations.

The competent statistical study of this question began in 1893 with the paper by Messrs. King and Newsholme. Mr. George King, one author, was Secretary of the Institute of Actuaries and Dr. Arthur Newsholme, the other, was the author of the standard English treatise on vital statistics. This article, written in coöperation by men both adequately equipped in statistics and one equipped in medicine, is probably the most significant statistical discussion of the problem which appeared before 1915 and therefore I shall begin with an examination of its arguments in the light of the additional evidence made available since it was written.

To prove that there is no increase in cancer mortality, all of the apparent increase must be explained by an appeal to other causes. Messrs. King and Newsholme were the first students of the problem who trod this difficult road and they have not found imitators. It is in their choice of this method rather than in the conclusiveness of their results that the main value of their paper lies.

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[5] King and Newsholme, p. 228.

[6] Hoffman, p. 218.

Their main conclusion and the one with which we are primarily concerned was that "the increase in cancer is only apparent and not real." Their argument shows that this statement refers only to cancer mortality and is in no wise inconsistent with an increase of cancer morbidity offset by a diminishing case fatality.

Their subsidiary conclusions supporting this main conclusion were two:

1. A part of the increase in the crude cancer mortality, that is, the deaths from cancer per 100,000 population of all ages, is due to the increasing average age of the population and is eliminated when standardized death rates, or rates in a population of standard age composition, are computed.

2. In Frankfort-on-the-Main, the only population group in which the annual budget of deaths from cancer at each age and for each sex had long been carefully classified by the organs or parts of the body primarily affected, there was no increase in the mortality from accessible cancer, but a marked increase in the mortality from inaccessible cancer. This was thought to be conclusive evidence that the increase was due to better diagnosis, or, in the writers' words at the close of the communication,

> The increase in cancer is only apparent and not real and is due to improvement in diagnosis and more careful certification of the causes of death. This is shown by the fact that the whole of the increase has taken place in inaccessible cancer difficult of diagnosis, while accessible cancer easily diagnosed has remained practically stationary.[7]

This argument was apparently regarded by the authors as decisive and upon it they rested their case.

Their argument thus is twofold: part of the increase is due to changes in the age and sex composition of the population and eliminated by the method of correcting the death rate; the rest of it is due to improved diagnosis and eliminated by confining attention to cases of accessible cancer in which there has been little room for improved diagnosis.

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[7] King and Newsholme, p. 228.

The second of these two main arguments was supported by two minor arguments, which may be first reviewed in the light of the new evidence.

1. The increase of cancer mortality between 1860 and 1891 among men insured in the Scottish Widows' Fund Life Assurance Society, the only group of insured persons for which the requisite information could be obtained, was much less rapid than the increase in the general population of Scotland. This difference was explained by supposing that when the figures began, the diagnosis of cancer among the insured persons, who as a class were well-to-do, was more accurate and, in consequence, the effect of improvement of diagnosis during the thirty years was less among the insured than in the general population.

2. Cancer mortality among men was lower but increasing faster than among women. This was ascribed to the greater proportion of cases of inaccessible cancer among men, the greater obstacles to accurate diagnosis in such cases, and the more rapid improvement in the diagnosis of inaccessible cancer.

Their argument from insurance experience is illustrated by the following figures compiled from those in their article.

Death rate from cancer per 100,000 males at least twenty-five years of age, distributed in age groups as in English Life Table No. 3

PERIOD	INSURED IN SCOTTISH WIDOWS' FUND LIFE ASSURANCE SOCIETY	IN TOTAL POPULATION OF SCOTLAND
1860–1866	60	70
1867–1873	83	82
1874–1880	75	101
1881–1887	85	115

The increase of the death rate among the general male population was 45 per 100,000, or 64 per cent, that among the insured males was 25 per 100,000, or 42 per cent. Mr. Hoffman quotes some additional evidence furnished by the medical officer of the same Society,[8] the figures of which, it will be noted, do not agree entirely with those published by Messrs. King and Newsholme. I learn from the Actuary of one of the largest American insurance companies that probably no evidence on this point has been published by any American company. But even if it were buttressed by much additional material, I cannot attach great weight to this argument, because of the uncertain influence of selection in any class of insured persons. During the thirty years covered by the experience of the Scottish insurance company, for example, it may have extended its business among classes somewhat less subject to cancer than its earlier policy holders or the protective influence of the company's medical selection may have diminished.

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[8] Hoffman, pp. 84, ff.

The second argument advanced by Messrs. King and Newsholme is based upon the lower rate and more rapid increase in the cancer mortality of men. Among males the cancer usually attacks an inaccessible organ or part of the body and for that reason is difficult to diagnose correctly even at the time of death, a difficulty which has decreased with the improvements of modern medicine and surgery. Thus, accepting as correct their classification of deaths from cancer in Frankfort into the two groups of accessible and inaccessible, 97 per cent of the male deaths and only 63 per cent of the female deaths ascribed to such cancers

Death rate from cancer per 100,000 persons of sex indicated and at least twenty-five years of age distributed in age groups as in English Life Table No. 3

COUNTRY	1860	1890	INCREASE	
			Amount	Per cent
Males				
England	59	145	86	146
Scotland	67	174	107	160
Ireland	59	92	33	56
Females				
England	119	210	91	77
Scotland	116	220	104	90
Ireland	72	111	39	54

occurred where the seat of the disease was inaccessible.[9] Their argument is illustrated by the foregoing figures on the preceding page computed from those in their paper.

The foregoing figures show that in all these divisions the female rates at both dates were much higher, that in England and Scotland the actual increase for the two sexes is about the same and the relative increase for men much greater, but that in Ireland the same facts do not appear. The following question may then be addressed to the material in the recently published compilations. Is the cancer mortality of men lower but increasing faster than that of women?

Mr. Hoffman is the only one of these authorities to give death rates by sex. He gives them for each of the New England states, for Massachusetts since 1856, for Rhode Island since 1871, for Connecticut since 1879, for New Hampshire since 1887, for Maine since 1892, and for Vermont from 1871–1896. In two of these states the absolute increase and in four the relative increase of cancer mortality was greater among men than among women. A summary of his figures for the first five states since1896 by sex yields the following results:[10]

PERIOD	DEATH RATE PER 100,000 POPULATION		INCREASE				RATIO OF MALE RATE TO FEMALE (= 100)
			Amount		Rate		
	Male	Female	Male	Female	Male	Female	
1896–1900	46.2	90.3					51.2
1901–1905	53.6	100.9	7.4	10.6	16.0	11.7	53.1
1906–1910	61.5	110.9	7.9	10.0	14.7	9.9	55.5

These figures show that the rate of increase in cancer mortality among men has been somewhat more rapid than among women and that the ratio of male mortality to female increased 4.3 per cent in ten years.

For the ten states and the District of Columbia which have been included in the Federal registration area since the annual mortality reports of the Bureau of the Census began with 1900,[11] the cancer death rates by sex were:

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[9] Appendix, table 8.

[10] For the figures on which these rates are based see Appendix, table 1.

YEAR	DEATH RATE PER 100,000 POPULATION		INCREASE				RATIO OF MALE RATE TO FEMALE (= 100)
			Amount		Rate		
	Male	Female	Male	Female	Male	Female	
1900	47.0	80.7					58.2
1915	72.3	111.9	25.3	31.2	53.8	38.7	64.6

indicating that among men the increase was less, but the rate of increase greater, than among women The ratio of male mortality to female increased 6.4 per cent in fifteen years.

Among the twenty-six American cities, tables of which are included in his volume, there are ten in which the absolute increase and nineteen in which the relative increase of the cancer mortality is greater among men. The figures by sex since 1891 are given for fourteen American cities, Boston, Springfield (Mass.), Providence, Hartford, New Haven, New York, Philadelphia, Washington, Richmond, Augusta, Cleveland, Cincinnati, St. Louis and Nashville. They yield the following summary.[12]

PERIOD	DEATH RATE PER 100,000 POPULATION		INCREASE				RATIO OF MALE RATE TO FEMALE (= 100)
			Amount		Rate		
	Male	Female	Male	Female	Male	Female	
1891–1895	39.4	71.9					54.9
1896–1900	45.8	77.7	6.3	5.8	15.9	8.1	59.0
1901–1905	52.1	85.8	6.4	8.1	14.0	10.4	60.8
1906–1910	61.8	95.5	9.7	9.7	18.7	11.3	64.7

Thus, the cancer mortality of men in American cities has increased faster than that of women and the change has gone on more rapidly than in the American states. In fifteen years the ratio of male mortality to female has increased 9.8 per cent.

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[11] These states are Maine, New Hampshire, Vermont, Massachusetts, Rhode Island, Connecticut, New York, New Jersey, District of Columbia, Michigan and Indiana. They will be referred to hereafter as the registration states of 1900. See Appendix, table 2. For our problem the figures of "the registration area" are without value, because that area has rapidly expanded since 1900.

[12] Appendix, table 3.

Among the thirty foreign countries for which Mr. Hoffman gives death rates by sex, there are twelve in which the absolute increase and twenty in which the relative increase of cancer mortality is greater among men than women and in the minority of ten are five small Australasian divisions. He gives the figures by sex since 1895 for England and Wales, Ireland, Norway, Bavaria, Italy, New South Wales, Victoria, South Australia, Queensland, Tasmania, New Zealand, Bermuda and Jamaica. By combining the results the following summary has been obtained.[13]

PERIOD	DEATH RATE PER 100,000 POPULATION		INCREASE				RATIO OF MALE RATE TO FEMALE (= 100)
			Amount		Rate		
	Male	Female	Male	Female	Male	Female	
1896–1900	57.2	76.9					74.4
1901–1905	63.8	82.3	6.6	5.4	11.5	7.0	77.5
1906–1910	72.2	89.4	8.4	7.1	13.2	8.6	80.8

In this case the tendency of the male rate to overtake the female is more clearly marked, for the amount as well as the rate of increase is faster among the men. The ratio of male mortality to female increased 6.4 per cent in ten years. As each rate in the table is based upon more than 100,000 deaths from cancer, the steady decrease in the difference between the sexes is of more importance for the argument than that shown in the preceding tables.

Among the thirty-three foreign cities for which Mr. Hoffman gives the mortality by sex there are twenty-four in which the absolute increase and twenty-six in which the relative increase of the cancer death rate is greater among men. The figures by sex since 1896, given for 13 foreign cities separately and the cities of Denmark collectively, yield the following summary.[14]

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[13] Appendix, table 4.

[14] Appendix, table 5.

PERIOD	DEATH RATE PER 100,000 POPULATION		INCREASE				RATIO OF MALE RATE TO FEMALE (= 100)
			Amount		Rate		
	Male	Female	Male	Female	Male	Female	
1896–1900	80.4	107.6					74.7
1901–1905	90.3	115.0	9.9	7.4	12.3	6.9	78.5
1906–1910	99.4	120.1	9.1	5.1	10.1	4.4	82.8

The ratio of male mortality to female has increased 8.1 per cent in ten years.

All the new evidence supports the conclusion of King and Newsholme that the death rates from cancer in the two sexes are approaching equality as a result of the lower rate and more rapid increase among men.

Having reviewed the new evidence in these two subsidiary arguments, we turn now to the second of the main arguments advanced by Messrs. King and Newsholme, an argument supported by this difference between the cancer mortality of the two sexes. This alleges that the increase in cancer mortality is confined to mortality from inaccessible cancer. Their evidence on this point is derived from the figures of Frankfort-on-the-Main, the only region in which both the population and the deaths from cancer had for many years been classified by age and sex and the deaths classified also in detail and with much care according to the part of the body attacked by the disease. They seem to have accepted the Frankfort returns without any attempt to investigate the accuracy of the classification according to the part of the body affected by a study on the ground both of the original material and of the methods of treating it, a precaution which might well have been taken when so much depended on its accuracy. The able statists in charge of the work in Frankfort probably did not attach to this classification as much weight as the English interpreters. Certainly they abandoned it in 1891 and the Frankfort director, in a letter to me on the subject, volunteered the statement that in the opinion of German statists the diagnosis of cancer was not entirely trustworthy and that physicians out of regard for the family sometimes certified a death really due to cancer to be due to some less distasteful cause, like tumor.

Notwithstanding these reasons for giving less weight than our authors do to the Frankfort figures, it seemed wise to bring them down to date, if possible. To do so was the more important because in the twenty-four years since their article was published little new evidence has appeared dealing thoroughly with the question on the basis of classifying the deaths from cancer into two groups, one in which the disease was in an accessible location and the other in which it was not. My first attempt was to bring the Frankfort figures down to date from the printed sources which had been used by Messrs. King and Newsholme. This revealed the fact that after 1891 the Frankfort data were not published in the earlier form and that in consequence their study could not be continued in this way.

To learn whether the needed information was to be found on the manuscript death returns of Frankfort and could be compiled at reasonable expense, I opened correspondence with Dr. Busch, director of the municipal statistics of that city, to whose assistance I am greatly indebted. He arranged to have subordinates in the Frankfort office continue the earlier form of tabulation until the current year, and the results were furnished to me in manuscript. The details of the tabulation were arranged by Dr. R. M. Woodbury, then a travelling Fellow of Cornell University pursuing his studies in economics and statistics at the University of Berlin. He went twice from Berlin to Frankfort for the purpose of explaining exactly what was wanted and of reviewing the results, and his services, both in Germany and later in Ithaca, were of much help in this part of the problem. In order to be sure that the later Frankfort figures were strictly comparable with the earlier, the returns for 1888 and 1889, which had been compiled by Messrs. King and Newsholme, were recompiled from the printed sources and thus their treatment of a few doubtful groups ascertained and followed.[15]

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[15] The needed sum was given by Dr. James Douglas at the suggestion of Prof. James Ewing, at whose request my paper was begun and who has aided me throughout its progress. Without his encouragement I should hardly have

Some writers have objected to the classification of deaths from cancer according as the seat of the disease was accessible or inaccessible, alleging either that a third class of intermediate cases should have been recognized or that certain organs or parts treated by King and Newsholme as accessible should have been put in the inaccessible class or vice versa. In reply to the first objection, it is clear that the introduction of a third class of intermediate cases would obscure the argument. For our purposes a dual classification with a few border line groups thrown to one side or the other according to where most of the cases in the group belong is far better than the alternative triple classification. Whether their classification could have been improved is a question of technical medicine into which fortunately there is no need to go. But, in passing, I may mention that their decisions have found remarkable confirmation in the replies made by thousands of American doctors to a circular letter of inquiry sent out by the Federal Census Bureau. Each physician was asked whether he was certain or uncertain of his own diagnosis of cancer in a specified case of death from that disease occurring in 1914 wherein he had certified the cause of death. To this point I shall recur later (pages 324 ff). My present object is their material down to date, and for that purpose I would have merely to bring to follow their classification, even if I knew, as I do not, that it had serious defects.

The resulting tables extend the original ones so that they embraces the fifty-four years, 1860–1913, instead of only the thirty years, 1860–1889, and nine thousand deaths instead of three thousand.[16] The final result is shown in the following table.

ventured so far into the field of medical statistics. The figures for 1860 to 1889, inclusive, are in the earlier article; those for 1890 are in the Stat. Mittheilungen of Frankfort for that year. But some of the certificates for 1890 and 1896 are now lacking and hence the returns for those two years have been disregarded. The manuscript tables obtained from Frankfort have been deposited with the Library of the Surgeon-General's Office in Washington, D. C., where they may be examined by any persons desiring to test my inferences from them. In the Appendix, tables 6 to 10, I have introduced all of King and Newsholme's Frankfort material, each table supplemented by additional figures so as to add five new periods between 1888 and 1913.

[16] Compare Appendix, table 10.

*Frankfort. Annual deaths from cancer in 1,000,000 living, at least twenty-five years of age. Population distributed in age groups according to English Life Table No. 3—Persons**

PERIOD	ACCESSIBLE	INACCESSIBLE	POSITION UNDEFINED	TOTAL
		Males		
1860–1866	126	1,118	359	1,603
1867–1873	88	1,421	137	1,646
1874–1880	14	1,913	363	2,290
1881–1887	35	1,865	305	2,205
1888–1893†	91	2,289	171	2,551
1894–1899‡	66	2,489	104	2,659
1900–1904	51	2,742	118	2,911
1905–1909	68	2,791	119	2,981
1910–1913	119	2,522	130	2,771
		Females		
1860–1866	1,081	1,323	293	2,697
1867–1873	1,214	1,540	254	3,008
1874–1880	1,220	1,588	131	2,939
1881–1887	981	1,820	272	3,073
1888–1893†	1,275	1,959	174	3,408
1894–1899‡	903	1,936	122	2,961
1900–1904	914	2,013	115	3,042
1905–1909	925	2,311	180	3,416
1910–1913	913	2,057	152	3,122

* Figures from 1860 to 1887, inclusive, derived from King and Newsholme, table XVI. Figures for later years derived from manuscript tables from the Frankfort Statistical Office.

† Omitting the year 1890, for which the figures were not available.

‡ Omitting the year 1896, for which the figures were not available.

The general meaning of the table can be read more easily and clearly in the form of a diagram.[17] For the sake of simplicity the insignificant categories of "position undefined" for each sex and the total rates for each sex have been omitted and the diagram depicts, therefore, the conditions stated in the first two columns of the preceding table.

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[17] See p. 281.

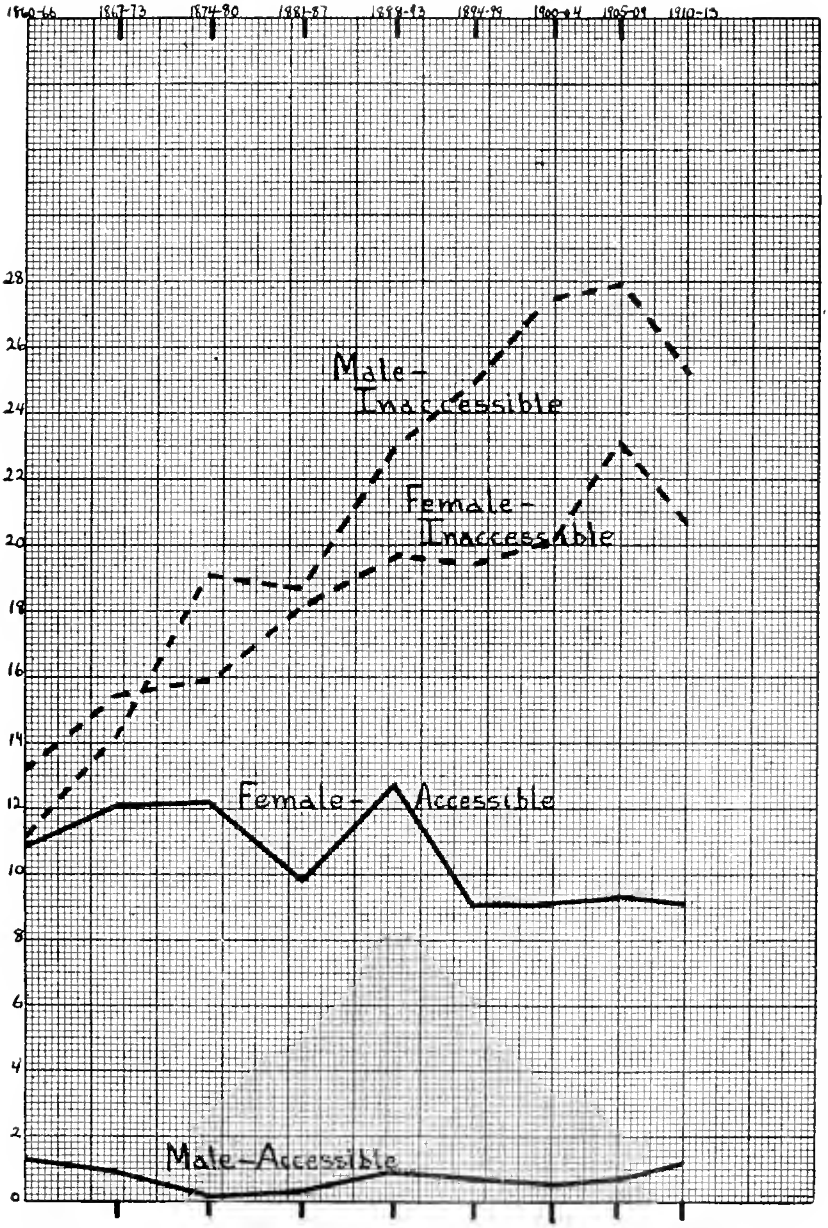

DIAGRAM I. Death Rates from Cancer, Accessible and Inaccessible, Frankfort-a-M.: 1860–1913.

Both table and diagram show, each in its own way, that, when the increase of cancer mortality by sex is analyzed into the increase from accessible and the increase from inaccessible cancer, there has been no significant change in the small mortality from accessible cancer of men and there have been irregular changes followed by a remarkable uniformity in the mortality from accessible cancer of women. Almost all the increase occurred in the mortality from inaccessible cancer, although by a surprising exception that mortality in each sex decreased between the last two periods, while the mortality of men from accessible cancer increased. The argument of Messrs. King and Newsholme from the Frankfort figures is thus materially strengthened.

To this argument several objections have been made.

1. "It is quite doubtful whether all uterine or even vaginal cancer can be accurately diagnosed as such without an exploratory operation or a microscopical examination of the diseased parts."[18] That American doctors as a group think differently appears clearly from the table on page 326.

2. "A fair proportion of deaths from cancer are those of the skin, other parts of the mouth than the tongue, and other external parts of the body"[19] and these should have been classed as accessible. The objection overlooks the fact that these classes are not distinguished in the printed sources used by Messrs. King and Newsholme.

3. The authors "for reasons unknown" have not reëxamined the Frankfort data since 1893.[20] This overlooks the fact, already mentioned, that the method of publishing the Frankfort figures was radically changed at about the time the original article was published and serious difficulties put in the way of a reëxamination.

4. It is not clear whether correction was made for deaths from cancer among inmates of Frankfort hospitals who were non-residents of that city.[21] Careful inquiry at the Frankfort

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[18] Hoffman, p. 31.

[19] Ibid., p. 31.

[20] Ibid.

[21] Ibid., footnote.

registration office, supplemented by inspection of the manuscript returns, showed that they were not systematically excluded, but that their small number made them unimportant.

5. The classification of deaths in Frankfort "fails to conform to modern requirements in that it is not in accordance with the international classification of causes of death."[22] A classification is to be judged with reference to the object it was designed to serve and the character of the material to be classified. There is little reason to suppose that the international classification is better adapted to the specific study of cancer death rates than another which may have had that object in view and certainly has been found valuable for it.

6. The results do not agree with those derived from the Frankfort data for 1906–1913 given in the official reports of the Frankfort Medical Society.[23] The latter are in conflict with those for the same years which I have derived from the official records of the Frankfort statistical bureau and already presented, and the differences appear irreconcilable. The figures of the Medical Society make no attempt at a classification into accessible and inaccessible and in consequence have little bearing upon the argument.

7. The standardized death rates from cancer in Frankfort, 1860–1889, by five periods of years show irregular changes, perhaps due to paucity of numbers, but they cannot be interpreted as affording no evidence of increase in the rates for accessible cases among women.[24] To this objection the supplementary figures already presented afford perhaps a sufficient reply.

These objections to the argument of Messrs. King and Newsholme do little to weaken its probative value. An objection of greater weight than these lies against their reliance upon standardized rates and will have to be considered later. In face of all the objections we can hardly accept their conclusion in the emphatic way in which it is stated. Neither the original argument nor my amplification of it, nearly doubling the period of observation and more than trebling the number of cases, can be held to justify completely their statement that "the increase in cancer is only apparent and not real and is due to improvement in diagnosis and more careful certification of the causes of death." The great merits of their paper lie in exemplifying the correct method of attacking the problem and showing beyond question that a measurable part of the increase in the mortality from cancer is due to a better diagnosis of the causes of death and another measurable part to the increasing proportion of persons who live on to ages when cancer is prevalent.

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[22] Hoffman, p. 33.

[23] Ibid., p. 31.

[24] Greenwood and Wood, On Changes in the Recorded Mortality from Cancer and their Possible Interpretation, in Proceedings of the Royal Society of Medicine, vii, no. 1, pp. 119–70.

Let us now examine the first of their two main arguments, namely, that part of the increase is due to changes in the age and sex composition of the population and that this part can be eliminated by the use of standardized death rates. No one will question that the increasing proportion of elderly persons in the population accounts for part of the increase in cancer mortality. But that this part can be eliminated by the help of standardized rates in dealing with the problem has been somewhat uncritically taken for granted in the United States and England. Various earlier writers had pointed out that cancer is preeminently a disease of adult and elderly life and that the proportion of the population at any moment living at ages when cancer is frequent is increasing with the lengthening of human life. But it was reserved for Messrs. King and Newsholme to put the argument into the necessary quantitative form. The method employed calls for a word of description. First, some population is selected as a standard; it may be the population of a country like that of England and Wales in 1901, which has been used in certain English and American computations, or an ideal or imaginary population, like that developed in a life table. Messrs. King and Newsholme chose the latter alternative and employed the population indicated by English Life Table No. 3, Persons, as a standard. Secondly, the number of each sex and at each age in each million of the standard population, or what is called the standard million, is computed. Thirdly, the death rate from cancer at each age and of each sex per 100,000 living at that age and of that sex in the population studied is found for each of the two dates, and the population at that age and of that sex in the standard million multiplied by these two rates. Fourthly, these sets of computed deaths are added, giving a total which shows how many persons would have died of cancer at each date in a standard million each group of which was subject to the death rates prevailing in the corresponding group of the observed population at the beginning and the end of the period observed. The increase or decrease in these sums of computed deaths is believed to show how much the cancer mortality in the population would have increased or decreased, if in the interval there had been no change in the sex and age composition, but just such changes as did occur in the cancer mortality for each sex and age period. This method of using standardized rates has long been followed in England, has recently been adopted in the United States and is regarded by Messrs. King and Newsholme as a prime requisite to sound work in dealing with the present problem. They say: "In order to be useful, the materials . . . . must be presented in such form that, at any rate for several intervals of years, the deaths from cancer and the population may be grouped according to sex and age;"[25] and it was because these conditions were satisfied nowhere but in the Frankfort statistics and those of the Scottish Widows' Fund Life Assurance Society that our authors relied so heavily upon that material. Their principle is a veritable bed of Procrustes. Its acceptance excluded at one stroke probably all other cancer statistics than those mentioned, and, if it is sound, the significant material is still so meager as to make an extensive study even now quite out of the question. Accordingly the principle should not be accepted without a more careful scrutiny than it has hitherto received. The method assumes that a standard population is needed but that it is a matter of indifference what standard is adopted. Thus Messrs. King and Newsholme say: "It is not of importance what standard is selected, because, all the observations being treated alike, the comparisons instituted between them will be entirely trustworthy."[26]

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[25] P. 212.

It is desirable to put this assertion to the test. The standards most likely to be in the mind of Englishmen writing in 1893 were the English Life Table No. 3 and the population of England in 1881, the date of the latest available census. A comparison of these two standards yields numerical results which are stated in the Appendix in the form of a standard million[27] and are expressed to the eye through the diagram on page 287.

The diagram shows clearly what the figures confirm and make precise, that the proportions actually living at the various ages in England in 1881 were very different from those shown by a life table. The life table assumes a population exempt from the disturbing factors of emigration and immigration and also from the distortion engendered by a rapidly increasing population, which swells inordinately the contingents belonging to the early years of life. Cancer is a cause of death almost unknown in infancy and childhood but common in adult and elderly years. Hence the standardized death rate from cancer obtained by using a standard in which adults and elderly persons are numerous must be much above the rate obtained by using a standard in which they are relatively few. For example, the standardized cancer mortality of Frankfort, 1860–1866, was 107 per 100,000, if the standard used is the English Life Table population, but only 70, or about two-thirds as high, if the standard is the population of England and Wales in 1881.[28] To this objection it might be replied that the information needed is not the standardized rate in any single period but the increase between two periods and that the increase, absolute or relative, might be the same even though the rates compared were widely different. But the answer is unsatisfactory for two reasons: first, if the absolute increases were the same, the relative or proportional increases could not be; and, secondly, as the in-

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[26] Pp. 211–12.

[27] See Appendix, table 11.

[28] See Appendix, table 12.

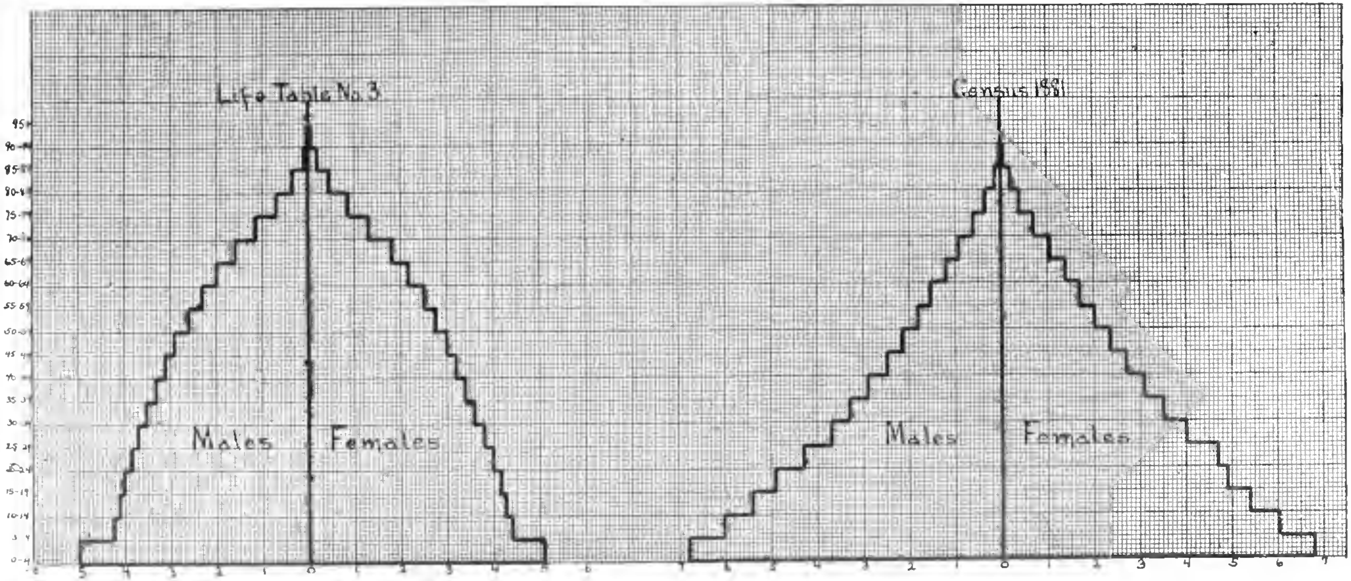

Diagram II. Standard Million, English Life Table No. 3 and England and Wales Census of 1881.

crease in the cancer death rate as well as its amount varies greatly with age, the chances against the agreement of two increases reached by using two such divergent standards are indefinitely great. In the case in hand, if the life table population is the standard, the increase in the standardized death rate of Frankfort is from 107 per 100,000 in 1860–1866 to 167 per 100,000 in 1888–1889, which is 60 per 100,000, or 57 per cent; but if the population of England in 1881 be the standard, the increase is from 70 in 1860–1866 to 107 in 1888–1889, which is 37 per 100,000, or 53 per cent.[29]

From this argument it might be inferred that the method of standardized death rates, if not generally invalid, is at least inapplicable to the present problem. But that would be going too far. In most civilized countries the average age of the population is slowly increasing, due in part to the fall in the death rate, especially in early years, and in part to the fall in the birth rate. For example, in the United States the median age of the population increased by 7.3 years between 1820 and 1910,[30] or about four-fifths of a year in each decade; and similar, though less rapid, changes are probably occurring in many other countries. Wherever this change is in progress some part of the increase in cancer mortality is due to it. It is theoretically possible that the whole might be so explained. Under these circumstances either some estimate of the part due to that cause must be reached or our problem abandoned on the threshold. An escape from the dilemma may be found along the following path.

If the simple or crude death rates from cancer in a given population at two successive periods are known and also the sex and age composition of that population at both periods and its death rates from cancer by sex and age at the later period, then the death rates by sex and age at the end of the period may be applied to the population living in corresponding groups at the beginning. The sum of these products gives the number of deaths from cancer which would have occurred, if the composition of the population had not changed during the period but the death rates from cancer had changed just as they did. From this sum a "corrected" death rate and the increase during the period due only to the changes in reported cancer mortality may be derived. A comparison between this increase and that in the crude rates shows how much, if any, of the latter is due to a change in the sex and age composition of the population and how much to a change in cancer mortality.

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[29] Appendix, table 13.

[30] United States Bureau of the Census, Thirteenth Census, vol. 1, p. 309.

This method of measuring how much of the increase in the crude death rate from cancer is due to changes in the sex and age composition of the population during the period under observation has been applied to a few cases chosen at random.

a. The American registration states of 1900[31] include all states for which the death rate from cancer both in 1900 and 1910 is known. The crude death rate from cancer was 63.9 per 100,000 in 1900 and 83 in 1910, an increase of 19.1 per 100,000, or 29.9 per cent.[32] The standardized death rate in 1910, accepting the distribution of population in the same states in 1900 as the standard, was 81.3, instead of 83, an increase over 1900 of 17.4 per 100,000, or 27.2 per cent.[33] As 27.2 is 91 per cent, or about nine-tenths, of 29.9, it is evident that about one-tenth of the increase in the crude rate was due to the decennial change in the sex and age composition of the population.

b. In England and Wales the crude death rate from cancer was 84.3 per 100,000 in 1901 and 99.3 in 1911, an increase of 15 per 100,000, or 17.8 per cent.[34] The standardized death rate in 1911, accepting the distribution of population in England and Wales in 1901 as the standard, was 91.5, an increase over 1901 of 7.3 per 100,000, or 8.7 per cent.[35] As 8.7 is less than half of

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[31] See p. 275, footnote 11.

[32] See Appendix, table 14.

[33] See Appendix, table 15. The method here used differs from that of the Bureau of the Census (Mortality Statistics, 1911, p. 33) in two respects; first, the standard population is not that of England and Wales in 1901, but that of the American registration states in 1900, and, secondly, the crude death rates used are those of the calendar years 1900 and 1910 instead of 1901 and 1911.

[34] See Hoffman, p. 596, table 6.

[35] See Appendix, table 16.

17.8, it is evident that more than one-half of the increase in the crude rate between 1901 and 1911 was due to the decennial change in the sex and age composition of the population.

c. In England and Wales the crude death rate from cancer was 58.9 per 100,000 in 1881–1890 and 75.8 in 1891–1900, a decennial increase of 16.9, or 28.7 per cent.[36] The standardized death rate, 1891–1900, accepting the distribution of population in England and Wales in the decade 1881–1890 as the standard, was 74.8 per 100,000, an increase of 15.9 per 100,000, or 27 per cent.[37] As 27 is about fifteen-sixteenths of 28.7, it is evident that about one-sixteenth of the increase in England's crude death rate from cancer in the decade 1891–1900 as compared with the decade 1881–1890 was due to the decennial change in the composition of the population.

d. In New York City the crude death rate from cancer was 65.5 per 100,000 in 1900 and 78 in 1910, an increase of 12.5 per 100,000, or 19.1 per cent.[38] The standardized death rate in 1910, accepting the distribution of population in New York City in 1900 as the standard, was 76.6, an increase of 11.1 per 100,000, or 16.9 per cent.[39] As 16.9 is about eight-ninths of 19.1, it is evident that about one-ninth of the increase in the crude death rate of New York City between 1900 and 1910 was due to the change in the composition of the population.

e. In London the crude death rate from cancer was 93.7 per 100,000 in 1901 and 107.2 in 1911, an increase of 13.5 per 100,000, or 14.4 per cent.[40] The standardized death rate in 1911, accepting the distribution of population in London in 1901 as the standard, was 95.5, an increase of 1.6 per 100,000, or 1.7 per cent.[41] As 1.7 is less than one-eighth of 14.4, it is evident that more than seventh-eighths of the increase in the crude death rate of London between 1901 and 1911 was due to the change in the composition of the population between those dates.

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[36] Registrar-General of England and Wales, Supp. to 55th Ann. Rep., pt. I, p. xxviii; Supp. to 65th Ann. Rep., pt. I, p. xcix.

[37] See Appendix, table 17.

[38] See United States Census Bureau, Mortality Statistics, 1909, p. 156, and 1910, p. 103.

[39] See Appendix, table 18.

[40] Hoffman, p. 605, table 16.

[41] See Appendix, table 19.

The general result of these calculations is to show that a considerable part of the increase in the crude death rate from cancer, a part ranging in these five cases from 5 to 88 per cent and averaging about one-third, is due to changes in the sex and age composition of the population. From the evidence I conclude that on the average not less than one-tenth of the apparent increase[42] may be thus explained and that, wherever the birth rate as well as the death rate has been falling over a period of years and in consequence the proportion of adult and aged persons in the population has been rapidly rising, there the change in the sex and age composition of the population may explain a much larger part of the increase in the crude death rate from cancer.

The article of Messrs. King and Newsholme, then, is faulty at a point where it has not hitherto been criticized, its unhesitating reliance upon standardized death rates and its rejection of all material which does not lend itself to that method. It is valuable because of its emphasis upon the influence of improved diagnosis as a factor and even more significant because it pointed out that other causes competent to produce the result must be detected and measured by whoever denies that any part of the apparent increase of cancer is real.

Since their article was published a few other intensive studies have appeared, although none, I believe, indicates so firm a mastery of the method to be pursued and none carries out so thoroughly the distinction between accessible and inaccessible cases. The results of these may now be briefly summarized.

Massachusetts (1850–1895). In his first study Dr. Whitney concluded that "The increase is probably due to better diagnosis and registration and until the ratio of deaths over thirty years [sc. to all deaths at that period of life] has reached 8 to 9 per cent, which is shown by autopsies to be the true rate for cancer, it is not justifiable to speak of the increase as inherent in the disease itself."[43] But in a later article he admitted the existence of "a steady increase in the deaths [sc. death rates?] from cancer which cannot be explained by improved registration and more careful diagnosis."[44]

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[42] I select this fraction somewhat arbitrarily because it comes near the result for the American registration states and the smaller of the two results for England. Results for a great country are likely to be nearer the average than those for any city, however populous.

District of Columbia (1881–1900). Dr. Smith accepts the views of Messrs. King and Newsholme and believes that the evidence does not demonstrate any increase in the true death rate from cancer.[45]

Providence, R. I. (1856–1905). Dr. Chapin thinks it probable, "that there has been no real increase in those forms of cancer which are visible or the diagnosis of which is easily made," namely, cancer of the breast and cancer of the uterus; and that "the apparent great increase in the mortality from cancer is due to improved diagnosis and changes in the sex and age distribution of the population."[46]

Paris (1886–1905). Dr. Bertillon showed that, during these twenty years, the death rate from cancer of the mouth, breast and female generative organs hardly increased at all, but that from cancer of the stomach increased rapidly and that from cancer of the intestines more rapidly. In other words, the increase in the recorded death rate was practically confined to cases of inaccessible cancer.[47]

Breslau (1876–1900). Dr. Frief showed that the death rate from cancer of the breast and uterus had been stationary and the whole increase was due to an increase in cancer of the abdomen. He concluded that, when the needed precautions had been taken and the needed corrections made, no regular and decided increase in the death rate from cancer was demonstrable.[48]

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[43] Wm. F. Whitney, Alleged Increase of Cancer in Massachusetts, Shattuck Lecture before Massachusetts Medical Society, June 11, 1901, p. 20.

[44] Statistics of Cancer in Massachusetts, in Boston Med. and Surg. Journ., clxii (May 19, 1910), p. 673.

[45] C. A. Smith, District of Columbia Cancer Record, in Am. Med., iii (1902), pp. 916–19.

[46] City Registrar's Report, 1905, pp. 89–93.

[47] Annuaire Statistique de la Ville de Paris, xxv (1904), p. 202.

Utrecht (*1872–1902*). Dr. Astro showed that the death rate from those forms of cancer which were easily diagnosed fell to three-fourths of the initial rate, while the death rate from cancer of other forms more than doubled.[49]

Amsterdam (*1862–1902*). Dr. Van Konijnenburg concluded that it was impossible to decide with confidence how much of the increase in the recorded death rate from cancer was due to a real increase and how much to improved diagnosis.[50]

Netherlands (*1867–1909*). Director Methorst was unable to determine whether the increase of cancer was due to a greater prevalence of the disease or to greater accuracy in its diagnosis, the latter influence certainly accounting for a part of the increase.[51]

England and Wales. Dr. Stevenson in the 74th Annual Report of the Registrar-General, for 1911, published in 1913, made a careful and illuminating study of cancer death rates for recent years in England, but reached no conclusion upon the main question. In the following year Major Greenwood, Jr., and Frances Wood presented before the Section of Epidemiology and State Medicine of the Royal Society of Medicine a paper, already mentioned, On Changes in the Recorded Mortality from Cancer and their Possible Interpretation,[52] which was largely a discussion of the evidence presented and arguments suggested by Dr. Stevenson, and reached the general conclusion "that there is some truth in the popular opinion that the real incidence of cancer has increased."

Both of these English papers and no little current discussion seem to favor the view that the general question with which this article is concerned, Is the mortality from cancer increasing? can best be attacked by breaking it up into a series of subordinate questions like, Is cancer of the breast increasing? and answering the former only by finding the resultant of various and probably conflicting currents of change in cancer of various parts or organs. I cannot but feel that this is postponing attack upon the simpler statistical problem until a series of more difficult ones are solved. In other words, however unsatisfactory the present discrimination and reporting of cancer as a cause of death may be, and I believe them, as a rule, to be very unsatisfactory, they are probably much more accurate and trustworthy than the reporting of the organ or part of the body primarily affected by the disease.

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[48] Klinisches Jahrbuch, xii (1904), p. 154.

[49] Quoted in Van Konijnenburg, Mortalité par Cancer à Amsterdam, 1862–1902, pp. 14 and 11. Müller, Amsterdam, 1911.

[50] Ibid.

[51] International Statistical Institute, Bulletin XIX, pt. III, pp. 310–312.

[52] Royal Society of Medicine, Proceedings, vii, no. 1, pp. 119–170.

These are all the significant intensive studies of the increase of cancer which have come to my attention.

An incidental result of rejecting the method of standardized rates, for the reasons already assigned, is that it opens the door for that extensive study of the material in the recent international compilations to which we now turn our attention.

At the beginning a few words about the sources are needed. As already mentioned, three important compilations of international statistics and two of American statistics have been published since 1893. The first was issued by the French statistical office with the coöperation and aid of statistical offices in other countries.[53] It has appeared in two volumes, the first in 1907, giving material from the beginning of registration until 1905, the second and supplementary volume appearing in 1913 and containing data for the period 1901–1910. These volumes give the deaths but not the death rates from cancer in the following 22 countries for each year during the period specified. The records all together include 694 years.

With the aid of this material the annual death rates from cancer per 100,000 total population have been computed and are given in the Appendix.[54] In some instances they will be found to differ slightly from the rates given by Mr. Hoffman.

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[53] Statistique Internationale du Mouvement de la Population. Imprimerie Natl., Paris, vol. i, 1907; vol. ii, 1913.

[54] Appendix, table 20.

COUNTRY	PERIOD	NUMBER OF YEARS
England and Wales	1858–1910	53
Scotland	1855–1910	56
Ireland	1864–1910	47
Norway	1870–1910	41
Austria	1873–1910	38
Switzerland	1877–1910	34
German Empire	1892–1910	19
Prussia	1875–1910	36
Bavaria	1888–1910	23
Saxony	1892–1910	19
Würtemberg	1892–1910	19
Holland	1875–1910	36
Spain	1900–1910	11
Italy	1887–1910	24
Serbia	1892–1910	19
New South Wales	1875–1910	36
Victoria	1864–1910	47
Queensland	1879–1910	32
South Australia	1879–1910	32
West Australia	1891–1910	20
Tasmania	1891–1910	20
New Zealand	1879–1910	32

As I have found no reason to question the accuracy of March's figures, I must ascribe these differences to differences in classification. I believe the earlier and later figures for each country in March are comparable and that is the important matter.

The second international publication was issued by the municipal statistical bureau of Amsterdam[55] and gives for each city of at least 100,000 inhabitants for which the information could be had the number of deaths and the death rate from cancer in each year since 1880. This compilation includes the death rates from cancer for 52 cities and 1,600 years. Like the first, it was prepared with the aid of the local authorities involved and may be accepted as accurate enough to warrant the use here made of its figures.

The third source of international statistics is Mr. F. L. Hoffman's The Mortality from Cancer Throughout the World, published in 1916 and containing (pp. 306–775) several hundred pages of statistical tables. It is especially rich and valuable in tables for the United States (pp. 422–581) and other American countries, a side in which the European compilations are weak.

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[55] Statistique démographique des grandes Villes du Monde, 1880–1909. Two parts. Amsterdam, Johannes Müller, 1911, 1912.

In addition to these three international sources a volume of American mortality statistics[56] gives a large number of American death rates from cancer for each of ten successive years, 1900–1909, inclusive, and a special bulletin on deaths from cancer in the registration area, 1914, gives a thorough and valuable analysis of this material.[57] From these sources a great number of annual death rates from cancer for successive years can easily be derived.

Before inquiring how far, if at all, improvements in the diagnosis of cancer account for the increase in reported cancer mortality, it will be prudent to consider how statements of the cause of death are obtained.[58] Four ways have been distinguished. (1) The cause is assigned by a medical examiner authorized and required to perform an autopsy whenever it is necessary for confirming the diagnosis. (2) The cause is assigned by an examiner required to examine the dead body and to consult with the attending physician but not authorized to perform an autopsy. (3) The cause is assigned by the attending physician or, in case no physician attended the patient, by the midwife, next of kin, coroner or other person best informed regarding the matter. (4) The cause is assigned by whoever reports the death to the registration officer.

Of these four ways, the first would be the most accurate and may in future become general. At present it is nowhere used and in the existing state of public opinion it could hardly be introduced. It is approached in many European hospitals, and when one considers the change of public sentiment about autopsies during the last century, one hopes that within another century or less this method will prevail. The frequency of autopsies is connected with the development of hospital facilities and greater in cities than in the country. In fifteen large cities of Switzerland 26 per cent of the deaths in 1900 and in the city of Basel 34 per cent of the deaths in 1902 were followed by autopsies. Of the autopsies in Basel 86 per cent occurred in hospitals and only 14 per cent outside.[59] But at present this method of determining the cause of death is merely an ideal or counsel of perfection. For our purposes the fourth method is useless.

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[56] Mortality Statistics, 1909. Washington. Govt. Ptg. Office, 1912.

[57] Mortality from Cancer and Other Malignant Tumors in the Registration Area of the United States, 1914. This material was kindly furnished me in proof by the Census Bureau. I take this opportunity to express my deep obligation to that office for the uniform courtesy with which my requests for unpublished information have been granted.

[58] Cf. von Mayr, Statistik und Gesellschaftslehre, ii, §75 (Die Todesursachen), pp. 309–329; and Prinzing, Medizinisches Statistik, pt. 3, chap. 6 (Die Todesursachen), pp. 318–406.

Hence valuable statistics of deaths from cancer exist only for cities or countries in which the cause of death is regularly certified either by a medical examiner or by the attending physician. The value of these methods and the accuracy of the returns depend largely upon the proportion of deaths which are certified by physicians, upon the ability of the physicians to diagnose, and upon their willingness to certify, cancer as a cause.

A layman who reports a cause of death is almost sure not to ascribe it to cancer.[60] Hence the proportion of deaths ascribed to that disease is likely to depend in some degree upon the proportion of physicians in the population, but more closely upon the proportion of deaths reported by physicians to all deaths. If the proportion of physicians in the population is greater now than formerly, this fact might account for a part of the increase in the crude death rate from cancer.

The following figures[61] show that the proportion of physicians in Germany has increased.

DATE	NUMBER OF PHYSICIANS TO 100,000 POPULATION IN GERMANY
1876	32
1887	33
1898	44
1904	54

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[59] Prinzing, Medizinisches Statistik, p. 320.

[60] Compare pp. 301, 316.

[61] Prinzing, op. cit., p. 541.

That a similar change was in progress in England and Wales down to 1901 appears from the following figures.

DATE	NUMBER OF PHYSICIANS TO 100,000 POPULATION IN ENGLAND AND WALES
1881	58
1891	66
1901	70
1911	65

There is no trend in the United States similar to that in England and Germany.

DATE	NUMBER OF PHYSICIANS TO 100,000 POPULATION IN THE UNITED STATES
1880	171
1890	166
1900	174
1910	164

The proportion of physicians to population in this country is about three times as great as in Germany and more than twice as great as in England, but is not increasing. Probably much of the difference is due to differences in the method of enumeration and to the readiness with which American enumerators accept without verification the claims of persons of questionable standing to be physicians. No doubt the quality, if not the relative number, of American physicians has improved within a generation and as a result their ability to diagnose cancer by clinical means, while still far from adequate, is probably greater than it was in 1880. At that date the accuracy of diagnosis made by the lower levels of the profession is brought in question by the spelling upon certain death certificates sent to the Federal Census Office in that year, of which the following are examples: "Tecis, Spinalgitis, Colory in Phantum, Colriafontim, Cholor Rhear Infantum, Hasplmar, New Money fever, No fisian tendin, Struck in on the aire sells, Yaller ganders of the Liver, Unnowing, Know Knowen Cause."[62]

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[62] United States Census Office, Tenth Census (1880), xi, Mortality and Vital Statistics, pt. 1, p. xii

In no part of the United States, I believe, are death certificates classified to show the proportion in which the cause was assigned by a physician and the proportion may be no greater now than ten, twenty or fifty years ago. The rise in the level of the profession, however, diminishing the proportion of incompetent practitioners, a change which took place in some European countries at an earlier period, has led to a similar result, for an incompetent physician, like a layman, will avoid the mention of cancer on his certificate. But in several foreign countries the proportion of deaths certified by a physician has greatly increased of recent years. In Saxony in 1873 only 37 per cent but in 1906, 66 per cent of the deaths were reported with a physician's statement giving the cause.[63] In Bavaria the proportion of decedents who were attended by physicians and whose deaths were medically certified rose from 45 per cent for the years 1845–1850 to 65 per cent for the years 1901–1902. In Würtemberg it rose from 47 per cent for 1876–1881 to 63 per cent for 1899–1900; in Baden, from 52 per cent for 1852–1859 to 70 per cent for 1892–1901. In Switzerland the statistics of causes of death are perhaps better organized than anywhere else. Dr. Prinzing awards it this preëminence among the European countries.[64] Particularly important for our purposes are the provisions in Switzerland whereby the attending physician makes a confidential report upon the cause of death to the statistical bureau and issues another certificate as a prerequisite to the burial. It may be more than a coincidence that the death rate from cancer in Switzerland is much higher than in any other country, but has not increased since 1900.[65] Both of these facts it would be easy to harmonize with the theory that the reported death rate from cancer depends largely upon the accuracy of certification and its increase upon the improvement of certification. The proportion of medically certified deaths in Switzerland increased from 82 per cent in 1876–1880 to 96 per cent in 1901–1903. In these five countries as a group, and they are the only ones for which I have found the information, the ratio of deaths medically certified to all deaths has increased about 0.5 per cent yearly.

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[63] Georg Radestock, Zur Statistik der ärztlichen Beglaubigung von Todesursachen, in Zeitschrift des k. Sächs. Statistischen Landesamtes, liv (1908), pt. I, pp. 133–139.

[64] Prinzing, op. cit., pp. 321 and 325.

[65] Appendix, table 20, and Hoffman, p. 674.

If the person dying was in early adult years, a physician was probably in attendance. But if he was a nursing infant or aged and decrepit, the chances may be against it. The probability that a physician was in attendance varies in a measure with the economic value of the endangered life and that depends not merely upon the wealth or position of the family but also upon the age of the individual. Thus in Saxony in 1905 among children under one year of age less than two deaths in five were medically certified, but at ages between twenty and thirty about fifteen in sixteen were so certified. With advancing age the proportion sank rapidly: at 50 to 59 it was 91 per cent; at 60 to 69 it was 86 per cent; at 70 to 79 it was 71 per cent and at ages above 80 it was 55 per cent.[66] Similar variations with age are found in the figures for Würtemberg.[67]

The death rate from cancer steadily increases with age until near the end of life.[68] There is a large proportion of persons dying at an advanced age without a physician's care. Probably this proportion is decreasing with the progress of the years accompanying the decrease in the proportion at all ages already demonstrated and the improved economic position of the poor. These facts together furnish good ground for believing that a steady and important shift in the certification of deaths from an indefinite cause, like old age, to cancer has been in progress and that this change has been closely dependent upon that in the certification of deaths at high ages from certification by a layman to certification by a physician. The deaths in Saxony in 1905 were classified according to the cause assigned and the proportion under each cause which received medical certification was stated. At the top of the list was cancer, a cause certified by physicians in 97 per cent of the cases, and near the bottom of the list was old age, a cause certified by physicians in only 42 per cent of the cases. Hence the spread of medical certification would necessarily result in the appearance under cancer of not a few deaths which, under earlier conditions, would have been certified by a layman as due to old age.

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[66] Radestock, op. cit., p. 137.

[67] Prinzing, op. cit., p. 322.

[68] The highest death rate almost always occurs at some age period above 70. See Appendix, tables 12, 13, 15, 16, 17, 18, 19.

Influences contributing to the same result are the extension of hospital facilities and the increase in the proportion of autopsies. It is difficult to get figures about either. The importance of autopsy in diagnosing cancer is illustrated by Riechelmann's statement that in 1895–1901 a municipal hospital in Berlin had 711 cases of cancer established by autopsy, 156 of which, or 22 per cent, had not been so diagnosed during the lifetime of the patient, although the diagnosis had been made with the greatest care by the highest authorities, thus excluding the carelessness with which the ordinary physician in Berlin still reported the causes of deaths occurring in his practice.[69]

To be sure, not all the mistakes are on the same side. Sometimes a clinical diagnosis of cancer is negatived by the findings at the autopsy. But the additions resulting from autopsy exceed the subtractions. This is indicated by Dr. Bashford's evidence. In 1909 he reported upon 9488 fatal cases in hospitals which had been treated during the life of the patient as cancer and after death had been checked by autopsies. This check showed that 757 cases, or 8.0 per cent, of those so diagnosed were not cancer. But during the same period and in the same hospitals 1801 other cases of fatal disease, more than twice as many, which during life had not been diagnosed as cancer were shown by autopsies to be so. The net result was to show that the true number of deaths from cancer in these hospitals was 10,532 instead of 9488, so that the number diagnosed as cancer rightly or wrongly by clinical means during the lifetime of the patient was only 90 per cent of the deaths actually due to this disease. In cases of cancer attacking accessible parts of the body the number of deaths determined by clinical examination was 98 per cent of the true number; but where inaccessible parts of the body were primarily involved the number of deaths determined by clinical examination was only 68 per cent of the true number.[70]

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[69] W. Reichelmann, Eine Krebsstatistik vom pathologisch-anatomischen Standpunkt, in Berliner Klinische Wochenschrift, xxxix (August 4, 1902), p. 729.

Dr. Williams has suggested that "these were specially selected lots of difficult hospital cases and cannot be regarded as in any way typical of every day experience."[71] This is a question upon which a layman must speak with diffidence, but in my opinion the greater attention and skill bestowed upon hospital patients would more than offset the greater difficulty of diagnosis, if it exists.

If physicians attending hospital patients often fail to diagnose cancer, such failures must be far more common among physicians in general practice, in which case an increase in the ratio of deaths in hospitals to all deaths would mean an increase in the ratio of deaths ascribed to cancer to all deaths from it. This suggests the question, Is the ratio of deaths in hospitals to all deaths increasing? The only evidence for the United States is indirect. At the end of 1904, to every 100,000 people there were 87 patients in American public hospitals; at the end of 1910 there were 118, an increase of more than one-third in six years. During the year 1904, the number of patients treated in public hospitals was 1309 per 100,000; during the year 1910, it was 2124, an increase of nearly two-thirds in six years.[72]

In Greater New York the proportion of deaths in hospitals to total deaths has doubled in the seventeen years since the enlarged city was organized, rising from 16.3 per cent in 1898 to 32.9 per cent in 1915. In old New York the same change was in progress through an earlier period, the proportion of deaths in hospitals to all deaths rising from 15.4 per cent in 1889 to 22 per cent in 1897.[73]

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[70] Lancet, 1909, ii (Sept. 4), p. 694.

[71] W. R. Williams, The Natural History of Cancer, p. 54.

[72] United States Census Bureau, Benevolent Institutions, 1904, p. 32; Benevolent Institutions, 1910, pp. 48–49.

[73] See Appendix, tables 21 and 22.

German figures giving the number of beds in general hospitals, public and private, indicate a change almost as rapid. In that Empire in 1877 there were 166 hospital beds to 100,000 people and in 1910 there were 400, nearly two and one-half times the earlier ratio.[74]

The figures for Austria[75] point to a slower increase. Owing to differences in the classification of hospitals in the two countries, the following ratios are not comparable with those for Germany.

YEAR	HOSPITAL BEDS PER 100,000 POPULATION
1870	118
1880	124
1890	142
1900	179

In France the average annual number of beds in hospitals in the periods 1871–1880 and 1901–1905[76] may be compared with the population in the middle of each period. The results show an increase in beds per 100,000 people from 193 to 236 in about thirty years. Probably a similar change is going on in nearly every civilized country and there is little doubt that it has materially increased the chance of correctly diagnosing cancer as a cause of death.

The preceding evidence shows that in every European country for which the information was available the proportion of physicians to population and the proportion of deaths certified by physicians have increased; that in the United States, although neither is known to have risen, the average competence of physicians has probably increased; that in several European countries even now a large proportion of deaths at ages when cancer mortality is highest are not medically certified; that cancer is the cause of death least often stated by a layman; and that hospital facilities are more numerous and autopsies more frequent than ever before. With this evidence in mind, we are in a better position to weigh the arguments tending to show that improvements in the diagnosis and certification of cancer, which have long been in progress and are still continuing, explain at least in large part the increase in the reported mortality from this disease.

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[74] See Appendix, table 23.

[75] Prinzing, op. cit., p. 545.

[76] From Stat. annuelle des Instit. d'Assistance (1906), cited in Webb, New Dict. of Statistics, 1911, p. 298.

In order to decide what may reasonably be looked for in the mass of international cancer statistics which has accumulated since 1893, let us assume for a moment the extremely skeptical position of those who deny that the cancer death rate has actually increased and believe that, apart from increasing longevity of the population, the entire increase in the reported death rate is due to improved diagnosis. Let us ask what results traceable in the figures would probably appear as corollaries or consequences of this condition of affairs.

To simplify the problem, assume provisionally that the only influence affecting the death rate from cancer which has been statistically demonstrated is age, or, in other words, that there are no demonstrated differences in the true cancer death rate of men and women, of white and colored, of residents in city and country or in different countries, but that all differences in the recorded rates of these groups at the same age are due to differences in the accuracy of diagnosis. Assume, furthermore, that in most of the regions from which statistics are derived there has been a steady increase in the number or an improvement in the quality of medical men or both and an increasing interest and ability of the public to coöperate with them in improving the statistics of causes of death. If these assumptions were correct, they would probably entail the following consequences:

1. With the progress of the years the proportion of deaths the cause of which was reported unknown would decrease and the proportion ascribed to various well recognized diseases, such as cancer, would increase.

2. In a similar way the proportion ascribed to specific ill-defined or unsatisfactory causes, like tumor or old age, would decrease and the proportion ascribed to cancer would increase.

3. In countries or districts in which the proportion of physicians to population or of deaths certified by physicians to all deaths is low, the cancer mortality would be low and vice versa.

4. Probably the ability to diagnose cancer is less common among physicians in country districts than among those in cities and, at least in the United States, the average number of persons to each physician is greater in the country than in cities. The average distance separating physician from patient is far greater in the country. Under these conditions, and if there is no difference between city and country in the actual cancer mortality at a given age, the reported cancer mortality at each age period would be less in the country. But the larger proportion of aged persons in the country and the high mortality of the aged from cancer might counterbalance this influence and make the gross rates in the country as high as in the city. If these differences between city and country are decreasing, then the reported cancer death rate would rise faster in the country.

5. Probably the ability to diagnose cancer is less prevalent among physicians attending negroes. If so, the reported death rates from cancer would be less among negroes than among whites.

6. Whenever ignorant or careless certification in any form and to any degree exists, it is likely to affect more seriously not only the weaker economic classes, like the negroes in the United States, but also the age periods which are economically less valuable, like those near the end of a normal lifetime. Hence with improvement of diagnosis and certification, the resulting increase in the reported death rate from cancer would be especially apparent in the later age periods.

7. When deaths from cancer were divided into classes, according to whether the part of the body primarily involved made the case easy or difficult of diagnosis, the increase in the classes difficult of diagnosis would be at a higher rate and continue to a later date than the increase in the classes at the other end of the scale.

8. As about 97 per cent of the men and only 63 per cent of the women dying of cancer have cases in which inaccessible organs

are primarily affected,[77] the death rate of men from cancer would be lower and would increase faster than that of women.

9. As the reported cancer death rate in any district or class rose towards the assumed higher but stationary true rate, the increase in the reported death rate would slacken and ultimately disappear.

If these inferences from the initial assumption, or any of them, are found to be supported by inductive evidence from the figures, that fact would strengthen the argument that the increase in the mortality from cancer is only apparent. In the further analysis of the evidence attention will be directed especially to the American figures because the conditions affecting their significance are better known to an American student.

1. Is the proportion of deaths reported as due to an unknown or ill-defined cause decreasing? In the part of the United States for which significant figures exist, the registration states of 1900, the number of deaths ascribed to ill-defined or unknown causes[78] since 1900 has been steadily diminishing. In 1900 there were 13,116 deaths from this group of causes; in 1915 there were 1090, a decrease of 12,026, or 91.7 per cent. But these deaths are distributed to the several ages of life in somewhat the same proportion as other causes of death, while cancer is characteristically a disease of adults and aged persons. Hence it is fairer to exclude all deaths ascribed to ill-defined or unknown causes in which the decedents were less than thirty years of age. In 1900 there were in these registration states 6362 deaths of persons over thirty years of age which were ascribed to ill-defined or unknown causes; in 1915 there were only 520, or about one-twelfth as many. As the population increased, while the deaths decreased, the death rate from such causes fell still faster, from 74.9 per 100,000 population over thirty years of age in 1900 to 4.5 per 100,000 in 1915, or one-sixteenth of the earlier rate.

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[77] These proportions are derived from the Frankfort figures for the fifty-four years 1860–1913. See Appendix, table 8.

[78] In general terms this group includes every death which was said to be due to ill-defined organic disease, sudden death, or a cause ill-defined or not specified.

An estimate of the number transferred from ill-defined causes to all other causes may be reached in the following way. If the rate in 1915 had been the same as in 1900, the deaths above thirty years of age from unknown causes would have been 8696. The difference between that number and 520, or 8176, represents the number probably transferred from ill-defined and unknown causes to some specific cause. If we assume that these were transferred in a random way, or in the proportions which each of those causes bore to all causes except the ill-defined group, then 9.6 per cent of the 8176 transfers, or 784, went to cancer. The actual increase in the cancer death rate between 1900 and 1915 was from 144.3 to 202.3 per 100,000 population over thirty years of age, or 40.2 per cent. If these 784 estimated transfers were excluded, the increase would be only from 144.3 to 195.5, or 35.5 per cent. On these assumptions between one-ninth and one-eighth, 11.7 per cent, of the apparent increase was due to transfers from ill-defined causes to cancer. But in assigning causes of death the modern tendency is away from vague, indefinite causes to specific, definite causes. Hence, the gain by transfers to cancer has probably been greater than a mere chance distribution of the transferred cases would indicate. If so, more than one-ninth of the increase in the cancer death rate of the registration states of 1900 between 1900 and 1915 was due to a certification of deaths in 1915 as due to cancer which, under conditions existing in 1900, would have been returned as due to ill-defined or unknown causes.

In dealing with this aspect of the question, the specific conditions surrounding certification and registration in each country must be known, and especially whether the residual class of ill-defined and unknown has retained the same meaning if errors are to be avoided. This knowledge I have found it difficult to acquire and for that reason I have made no study of transfers in other countries from ill-defined and unknown to cancer and of their effect in raising the apparent increase of cancer.

But after this warning it may be pointed out that in England and Wales, according to March, the deaths ascribed to unknown causes fell from 45,273 in 1858 to 3318 in 1910, a decrease of

93 per cent. However, in this case there is reason to suppose that the meaning of the term *unknown* has changed.[79] In Scotland between 1855 and 1910 the deaths from unknown causes fell off by three-fifths and, as the population increased, the death rate fell off by three-fourths; in Ireland the death rate from unknown causes fell by one-third between 1881 and 1910. In Prussia between 1875 and 1910 the deaths from unknown causes decreased from 101,633 to 18,434, or nearly five-sixths and the rate about eight-ninths; in Baden the decrease in deaths from unknown causes was from 1381 in 1881 to 17 in 1910; in Italy from 22,977 in 1890 to 3455 in 1910 and in the more populous divisions of Australia since 1891 similar changes have been in progress with great rapidity. All this evidence seems to warrant us in concluding that in many countries the assignment of deaths to cancer, which under earlier conditions would have been assigned to unknown or ill-defined causes, has played an important part in exaggerating the increase in reported cancer mortality.

2a. Is the proportion of deaths reported as due to tumor decreasing? Another kind of transfer which has been in progress, at least in the United States, during the period of the observations and has exaggerated the real increase of cancer has been the diminishing confusion between cancer and tumor. The reported death rate from tumor, or, more exactly, benign tumor, has been decreasing, while the death rate from cancer, sometimes called malignant tumor, has been increasing. These changes in opposite directions are probably to be ascribed in large part, if not entirely, to a more accurate discrimination between cancer and tumor, or a greater willingness among friends of the decedent to have the word *cancer* appear on the death certificate. As a result many deaths in recent years have appeared in the cancer column which under earlier conditions would have appeared as tumor. American evidence regarding this change goes back to a period long before 1900. In the census of 1880, this statement appears: "The reported number of deaths from cancer . . . . is undoubtedly too small, if we use the term 'cancer' in the ordinary sense, for there is very little doubt that most of the deaths reported as due to tumor . . . . might properly have been classed with cancer."[80] Ten years later the same authority said: "As it is impossible from the census data to distinguish the cases of tumor from those of cancer, they will be considered together."[81] The same treatment was accorded to them in 1900.[82] The diminishing proportion that deaths ascribed to tumor made of all ascribed to cancer and tumor combined is shown in table 24 in the Appendix, from which the figures below have been derived.

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[79] March, I, p. 829 and II, p. 235. But see the divergent figures for 1901 under this head in the two volumes.

Per cent that deaths from tumor make of total from cancer and tumor

DATE	CENSUS RETURNS FOR ALL UNITED STATES
1880	12.0
1890	11.7
1900	10.4
	RETURNS FOR EXPANDING REGISTRATION AREA OF UNITED STATES
1900–1904	2.3
1905–1909	1.5
1910–1914	0.9

The first three lines refer to deaths from any part of the United States as returned by the enumerators outside, and by medical certification inside, of the registration area. The other three refer to the registration area alone, which embraced in 1900 two-fifths and in 1914 two-thirds of the population of the country.[83] This expansion has carried registration into districts where the diagnosis of cancer is less trustworthy and hence the preceding figures obscure the results of improved diagnosis in an unchanging area. An idea of the exaggerated emphasis upon tumor as a cause of death in the enumerators' returns alone may be derived from table 24,[84] by subtraction. It appears that outside of the registration area of 1900, and thus in districts where the only source of information was enumerators' returns, 22 per cent of the deaths from cancer and tumor were ascribed to tumor, but within the registration area at the same date only 2.9 per cent of the deaths in that group were ascribed to tumor.

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[80] U. S. Census Office, Tenth Census, xii, p. cxiv.

[81] U. S. Census Office, Eleventh Census, Vital Statistics, pt. I, p. 336.

[82] U. S. Census Office, Twelfth Census, iii, p. clxxxi.

[83] U. S. Bureau, of the Census, Mortality Statistics, 1914, p. 9.

[84] Appendix, table 24.

The evidence derived from the registration states of 1900 for the period 1900 to 1915 points to the same conclusion and is more weighty because the area covered by the figures did not change. In this case it is safe to speak of rates rather than of proportions. The results are as follows:

*Death rate per 100,000 population in registration states of 1900**

YEAR	CANCER	TUMOR	BOTH	PER CENT OF DEATHS FROM CANCER
1900	63.9	2.0	65.9	97.0
1901	65.8	1.6	67.4	97.6
1902	65.6	1.4	67.0	97.9
1903	69.0	1.7	70.7	97.6
1904	70.4	1.4	71.8	98.1
1905	72.4	1.4	73.8	98.1
1906	73.1	1.3	74.4	98.3
1907	75.7	1.2	76.9	98.5
1908	76.8	1.0	77.8	98.7
1909	79.4	1.0	80.4	98.8
1910	83.0	1.2	84.2	98.6
1911	83.9	0.8	84.7	99.1
1912	85.9	0.7	86.6	99.2
1913	88.7	0.5	89.2	99.4
1914	89.1	0.6	89.7	99.3
1915	91.8	0.5	92.3	99.4

* For the full table see Appendix, table 25.

In these fifteen years the death rate from cancer has increased 27.9 per 100,000 population, but the death rate from tumor has fallen 1.5 per 100,000, so that the death rate from both combined has increased only 26.4 per 100,000. Thus the transfer from tumor to cancer seems to account for more than one-twentieth of the total increase.

The influence of this progressive transfer of cases from tumor to cancer through a longer period is clearly revealed in the

Massachusetts figures.[85] As the state classification differs from the Federal and the latter is available only since 1900, I have relied throughout upon the former.

Death rate per 100,000 population in Massachusetts

PERIOD	CANCER	TUMOR	CANCER AND TUMOR	PER CENT OF DEATHS FROM CANCER
1853–1857	19.3	5.3	24.6	78.5
1858–1862	25.8	4.9	30.7	83.9
1863–1867	29.7	5.3	35.0	84.9
1868–1872	34.9	5.8	40.7	85.8
1873–1877	37.4	5.4	42.8	87.3
1878–1882	50.8	3.8	54.6	93.0
1883–1887	56.1	3.3	59.4	94.5
1888–1892	60.6	3.1	63.7	95.1
1893–1897	67.1	3.8	70.9	94.6
1898–1902	71.0	3.0	74.0	96.0
1903–1907	83.3	2.5	85.8	97.1
1908–1912	90.2	1.2	91.4	98.6
Increase or decrease(—)per 100,000	70.9	−4.1	66.8	

These figures indicate that about one-seventeenth of the increase in the cancer death rate of Massachusetts was due to the certification of deaths at the close of the period as caused by cancer which under earlier conditions would have been ascribed to tumor.

The only article I have met with in which deaths from cancer and tumor are separately tabulated is one dealing with the statistics of Breslau for the period 1876–1899.[86] From its figures I have compiled the following:

PERIOD	DEATHS FROM		PER CENT FROM TUMOR
	Cancer and tumor	Tumor	
1876–1879	666	94	14.1
1880–1884	969	67	6.9
1885–1889	1,291	69	5.4
1890–1894	2,024	204	10.1
1895–1899	2,729	307	11.3

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[85] For the full table see Appendix, table 26.

[86] F. Frief, Die in den Jahren 1876–1900 in Breslau vorgekommen Todesfälle an Krebs, in Klinisches Jahrbuch, xii (1904), pp. 133–200.

A comparison of these figures with the others suggests that many of the deaths in Breslau ascribed to tumor are probably due to cancer and that there has been no steady improvement in discriminating between the two causes of death such as is indicated by the American figures.

In England and Wales the Registrar-General in 1884 began to send an inquiry to every physician who reported a death without a satisfactory statement of its cause, and in 1887 extended this procedure to include every case of a death certified as due to tumor. In 1886 the deaths reported as due to cancer were 16,243, in 1911 they were 35,902, an increase of 18,659 in twenty-five years. But in 1911 there were 656 deaths which had been at first reported as due to tumor and which, after correspondence, were transferred to cancer. This number equals 3.5 per cent of the increase. Probably a large, perhaps an equally large, number of cases in 1911 were returned in the first instance as cancer, although under the conditions of greater carelessness or ignorance prevalent before 1887 they would have been reported as tumor. This English evidence seems to corroborate the American in indicating that a significant part of the increase in the cancer death rate of recent years, a part which may be conservatively estimated as one-twentieth, has been due to a transfer of cases from tumor to cancer.

2b. Is the proportion of deaths reported as due to old age decreasing?

In the American registration states of 1900 the number of persons reported as dying of old age is steadily and rapidly decreasing. In 1900 it was 10,015 and in the same states in 1915 it was 4380.[87] This change is not due to a decrease either in the total population or in those at high ages; for both the population and the proportion who die late in life to all deaths are growing. It is due rather to the unwillingness of many recording officials to receive vague statements of the cause of death and their efforts to secure on the death certificate a statement more specific and exact than "old age." Thus, in the Manual of the International Classification of Causes of Death, the vade mecum of American registration officers, the instruction under "old age" reads: "A very indefinite and unsatisfactory return as a cause of death. It is usually due to carelessness on the part of the physician and a more specific statement should be required." The decrease in the deaths ascribed to "old age" is due, then, to the increasing care with which returns are made and the closer supervision of them by the registration officials, and the change is a rough index of the improvement of registration. Deaths which would formerly have been ascribed to "old age" now appear under some other rubric. Doubtless many of them appear under cancer and these transfers contribute to swell the increase in the recorded, as distinguished from the actual, cancer death rate.

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[87] Appendix, table 27.

It is possible to estimate the proportion of the increase in cancer mortality which can be thus explained. To do so it is necessary to estimate first the number of deaths transferred from old age to all other causes and then the proportion and number which have gone to cancer. For this it is probably best to assume a constant ratio between the number of deaths ascribed to old age and the number of deaths of all persons above sixty years of age. In the registration states of 1900 this proportion in 1900 was 9.9 per cent and in 1915 it was 3.2 per cent. If the per cent had remained unchanged, 13,697 deaths would have been ascribed to old age in 1915; the number actually ascribed to it was 4380. Hence it is probable that there were not far from 9317 deaths in 1915 which under earlier conditions would have been ascribed to old age but were ascribed to some other cause as a result of improvements in reporting causes of death. Of these 9317 deaths, the number transferred to cancer may be estimated by assuming that the proportion was equal to that which the deaths above sixty years of age from cancer make of the deaths at that time of life from all causes except old age. This would indicate that 9.3 per cent of the 9317 deaths, or 866, were transferred from old age to cancer. If these 866 deaths be subtracted from the total ascribed to cancer in 1915, the increase in the cancer death rate during the fifteen years 1900–1915 is reduced from 27.9 to 24.6 per 100,000. In other words, the transfer from old age to cancer serves to account for about 12 per cent of the entire increase. But if the transfers to cancer are due mainly to improved diagnosis, the tendency to change from old age to cancer must have been much greater than the tendency to change from old age to heart failure or general debility or some other meaningless term. If so, the transfers from old age to cancer in this instance must account for more than 12 per cent of the increase in cancer mortality.

The difficulties which prevented an international study of the transfers from ill-defined and unknown causes to cancer do not equally hamper a study of the transfers from old age. I have found no reason to suppose that the heading *old age* or its counterpart in the international classification, *senile debility* or *senility*, has materially changed its meaning. The decreasing death rate from this cause is shown in the following table, giving the death rate from old age for every census year since the record began. The data are taken from March.

Death rate per 100,000 population from old age, or senility, various European states, in the census years

YEAR OF CENSUS	ENGLAND AND WALES	SCOTLAND	IRELAND	NORWAY	SWITZERLAND	PRUSSIA	BAVARIA	BADEN	ITALY
1861	136	189							
1871	123	167	295						
1875				49		264			
1880					112	251			
1881	102	129	304	51				180	
1885						217		197	
1888					137				
1890						248	245	215	101
1891	101	124	375	47					
1895						221	213	182	125
1900				175	170	236	209	173	148
1901	93	96	287						
1905						201	191	154	143
1910	88	74	208	187	55	167	161	146	140

The preceding figures show that in most of the countries the death rate from old age is now falling and that in several it has fallen without interruption from the first. Probably in all these countries the proportion of deaths at ages above sixty, seventy or eighty to all deaths or to all persons living is increasing. Hence many deaths must be now ascribed to other causes which, under earlier conditions, would have been ascribed to old age, and no small proportion of these must have gone to cancer.

The preceding estimates explain away not less than three-tenths of the increase in the reported mortality from cancer, namely, transfers from ill-defined or unknown causes to cancer perhaps 11.7 per cent, transfers from old age to cancer perhaps 12 per cent and transfers from tumor to cancer perhaps 5.7 per cent. But the two larger figures have been reached by assuming that the transfers from indefinite to definite causes had a random distribution among the groups to which they went, while in fact the distribution is not random but tends to center on specific and precise causes. For this reason the results may fairly be deemed minimum figures and not a little below the truth. Accordingly, I conclude that from three-tenths to one-half of the increase in the death rate from cancer in the registration states of 1900 is due to the causes mentioned and is apparent rather than real.

If to these results we add the earlier conclusion that, on the average, not less than one-tenth of the increase in cancer mortality is due to the increasing proportion of adult and elderly persons in the population, I conclude that not less than four-tenths and perhaps as much as six-tenths of the increase in cancer mortality has been explained as only apparent.

These are all the results of improved diagnosis and certification upon the recorded cancer mortality which I have found any means of measuring even roughly. There are other changes, however, which have already been mentioned and need examination, although their influence cannot be measured.

3. Is the mortality from cancer low where there are few physicians or where a small proportion of the death certificates are prepared by physicians and vice versa?

To assume that few persons without medical training who had to certify the cause of a death would ascribe it to cancer might be safe. But on this point we need not rest with an assumption. In Saxony, of all deaths certified as due to cancer the per cent certified by laymen was 18 in 1876 and steadily decreased to 4 in 1903.[88] Hence, where certificates from doctors are few, the cancer mortality is likely to be low. In this respect no country, perhaps, includes greater extremes than Austria; Vienna is one of the medical centers of the world; Bukowina and Galicia are most meagerly furnished with physicians. Prinzing gives for each of the seventeen provinces of Austria in the five year period 1896–1900 the per cent of deaths the causes of which were certified by physicians,[89] and Hoffman gives the death rates from cancer, 1907–1911, in the same provinces.[90] As the two returns do not speak for the same period, I have sought for the per cent of medically certified deaths in the later years. The source[91] gives the information only for 1907 and 1910. From this material I have computed the per cents in the third column of the table on p. 417.

Among the many factors which influence the reported death rate from cancer in Austria, there is one, the proportion of deaths certified by physicians, so potent as largely and, where the differences are great, almost exclusively to determine the rate. Evidently the very low reported death rate from cancer in Dalmatia and Galicia is due to the few reports from physicians and not to the infrequent occurrence of cancer in those provinces. A comparison of the second and third columns in the table shows that the proportion of medically certified deaths in every province increased between the earlier and later dates. The increase in the Austrian death rate from cancer during the

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[88] Georg Radestock, Die Krebsterblichkeit im Königreich Sachsen in den Jahren 1873–bis 1903, in Zeitschrift des K. Sächsischen Statistischen Landesamtes, li, pt. II, p. 263.

[89] Op. cit., p. 326.

[90] Op. cit., p. 680.

[91] Oesterreichische Statistik. The figures were kindly furnished by the New York Public Library.

PROVINCE	DEATH RATE FROM CANCER PER 100,000 POPULATION 1907-1911	PER CENT OF DEATHS WHICH WERE MEDICALLY CERTIFIED	
		1896-1900	1907 and 1910
Salzburg	140	99.0	99.9
Upper Austria	136	98.2	99.3
Vorarlberg	125	96.3	98.4
Lower Austria	120	99.7	99.9
Tyrol	110	93.0	94.2
Bohemia	104	99.1	99.7
Trieste	99	98.8	99.9
Moravia	97	94.4	95.8
Styria	96	79.9	83.7
Corinthia	83	72.4	77.5
Silesia	67	70.1	74.7
Carniola	49	35.0	35.9
Görz and Gradiska	46	43.1	47.1
Bukowina	46	32.8	33.7
Istria	43	44.7	51.0
Galicia	33	26.5	27.6
Dalmatia	24	29.1	30.5
All Austria	79	68.0	68.5

same period from 69 to 78 per 100,000 was doubtless connected with this spread of medical certification.

The following table has been compiled from one in Hoffman[92] by arranging the countries of Europe in the order of the cancer death rate.

This suggests that in the countries of Europe the cancer death rates vary, as in the provinces of Austria they have been shown to do, in general agreement with the number and quality of the physicians, or the proportion of deaths which are medically certified. But I have found no figures showing the proportion of physicians to population or the proportion of medically certified deaths in these countries and therefore must content myself with raising the question without answering it.

4. Can any differences be traced between the amount or the increase of cancer mortality in city and country?

The cities of the United States having at least 25,000 inhabitants in 1900 had 232 physicians to every 100,000 people and the rest of the country had 153, or only two-thirds as many. Probably the number of visits made by a physician annually is greater

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[92] Op. cit., p. 594.

COUNTRY	PERIOD	DEATH RATE FROM CANCER PER 100,000 POPULATION
Denmark (cities)	1908–1912	142
Switzerland	1908–1912	124
Holland	1908–1912	106
Sweden (cities)	1907–1911	105
Scotland	1908–1912	103
England and Wales	1908–1912	98
Norway	1908–1912	97
German Empire	1908–1912	87
Ireland	1908–1912	81
Austria	1908–1912	80
France	1906–1910	76
Belgium	1908–1912	67
Italy	1908–1912	65
Roumania (cities)	1907–1911	62
Spain	1908–1912	52
Greece (cities)	1904–1908	52
Hungary	1908–1912	46
Portugal	1906–1910	22
Serbia	1905–1909	12

in cities than in the country and the quality of his training is better. The proportion of young adults with lives economically of high value is also greater in cities, and not a few invalids migrate to cities to avail themselves of medical and hospital advantages. All these circumstances would lead one to look for a higher cancer death rate in cities, if the assumption were correct that a direct and important connection exists between the number and quality of the physicians in a community, and its reported death rate from cancer. On the other hand, in country districts the proportion of aged persons peculiarly liable to cancer is larger. If cities show a higher death rate from cancer, therefore, and if there is no actual difference in the liability of city and country folk of like age to die of the disease, the fact would probably indicate that the more favorable age composition of the city population was outweighed by the larger number and greater ability of the city physicians. Now, in the cities of the registration states of 1900 the death rate from cancer in twelve of the sixteen years during the period 1900–1915 was greater than in the country districts, indicating that in the United States there may be a connection between the proportion of physicians to population and the reported death rate from cancer.[93]

Between 1900 and 1915 the death rate from cancer in the cities of the registration states increased from 66 to 91.3 per 100,000, or 38 per cent. Meanwhile the country rate increased from 61.4 to 92.8, or 51 per cent, a more rapid increase in the country rate. During the same period we may be sure from evidence already given that hospital facilities and the eagerness to use them increased quite out of proportion to population. Hence there can be little doubt that deaths from cancer occurring in a hospital are now a larger proportion of all such deaths than formerly they were. And it is almost as certain that the current of cancer patients from country districts to city hospitals is increasing. If so, and if there are no other real differences between the cancer mortality of city and country districts than those due to differences in the proportion of elderly persons, the more rapid increase of the reported death rate from cancer in the country shows that some change, perhaps improved diagnosis and certification, has progressed so much more rapidly in the country as to overbalance the growing drift of cancer patients from country to city.

The tables in the international publications already mentioned facilitate a comparison between cities and country districts in certain European countries. Thus for England the population and deaths from cancer since 1880 are given for Birmingham, Bradford, Derby, London and Liverpool by Falkenberg and for the whole country by Hoffman. By subtracting the urban totals from those for all England a rough division between city and country is reached. In the same way the rates for Edinburgh and Glasgow combined may be compared with those for the rest of Scotland, those for Christiania with those for the rest of Norway, those for Amsterdam, the Hague, Rotterdam and Utrecht with those for the rest of the Netherlands, and those for Vienna and Prague with those for the rest of Austria.[94] The computation shows that in sixteen out of twenty cases the crude urban rate was higher than that in the rest of the country and that in every country but England the increase in rate between 1881 and 1909, or in the case of Austria 1881 and 1908, was more rapid in the country districts, thus indicating that in Europe the difference between city and country has been decreasing. The figures for Austria are especially noteworthy. In 1881 the death rate from cancer in Vienna and Prague was 119.0, in 1908 it was 133. In the rest of Austria the rate in 1881 was 38.6, in 1908 it was 75.0. While the urban rate was increasing 12 per cent, the rural rate increased 94 per cent. In view of the other evidence which has been presented regarding conditions in the various provinces of Austria, it seems probable that nearly all of this increase in cancer mortality outside of the two capitals is due to improved diagnosis and certification.

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[93] Appendix, table 28.

Apparently, then, cancer mortality in cities, or at least in great cities, is usually higher but increasing more slowly than it is in country districts, so that in this regard the difference between city and country is decreasing. This is especially true wherever the initial differences between city and country are very wide.

5. Is the death rate from cancer among negroes less than it is among whites?

The greater longevity of the whites would result in a higher death rate among whites of all ages, even if there were no difference in the death rates of persons of the same age belonging to the two races. If the true cancer death rates of the two races at the same age periods are the same and medical attendance and certification among negroes are poorer than among whites, the reported negro death rate from cancer would be lower than among whites. Unfortunately the death rates by race are available for only six years, 1910–1915. They speak for the registration states of 1910 and embrace an annual average of over 39,000 deaths from cancer of whites and 900 deaths of colored, nearly all of them negroes. The results are as follows:[95]

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[94] For detailed figures, see Appendix, table 29.

DATE	DEATH RATES FROM CANCER PER 100,000 POPULATION CLASSIFIED BY RACE IN REGISTRATION STATES OF 1910		PER CENT THAT DEATH RATE AMONG COLORED IS OF THAT AMONG WHITE = 100
	Whites	Colored	
1910	76.8	52.6	68.4
1911	77.0	59.3	77.0
1912	79.8	59.3	74.3
1913	82.6	64.0	77.5
1914	83.5	65.4	78.3
1915	85.4	68.8	80.6
Per cent increase.	11.2	30.8	

The reported death rate of colored from cancer is from two-thirds to four-fifths that of the white, but is increasing faster, so that the difference between the races is diminishing. In view of the short period, the small number of negro deaths involved and the erratic figures, no great weight can be given to the results.

I have obtained similar figures for the same years for the registration states of 1900, a much smaller area (see page 322.) They have one advantage—they can be safely compared with figures for 1900.[96]

Here again it appears that the death rate from cancer among the colored is lower but increasing faster than among the white. The changes from year to year are irregular. A comparison of the two tables shows that the death rate from cancer is uniformly greater for each race in the smaller region from which the states recently added to the registration area are excluded,

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[95] For figures in full see Appendix, table 30.

[96] The figures for 1900 refer to the census year rather than to the calendar year and the area embraced differs from that of the registration states of 1900 by excluding Indiana. But these differences do not materially affect the significance of the comparison here made.

DATE	DEATH RATES FROM CANCER PER 100,000 POPULATION CLASSIFIED BY RACE IN REGISTRATION STATES OF 1900		PER CENT THAT DEATH RATE AMONG COLORED IS OF THAT AMONG WHITE = 100
	White	Colored	
1900	62.5	41.3	66.1
. . . . .	. . . . .	. . . . .	. . . . .
1910	83.4	60.5	72.5
1911	84.1	74.2	88.5
1912	86.2	71.5	83.0
1913	89.0	70.8	79.6
1914	89.4	72.1	80.6
1915	92.1	79.4	86.2
Per cent increase.	47.4	92.2	

as in the second table, a fact agreeing well with the theory that differences in the cancer death rates are due largely to the efficiency of the registration system, which even under the best of fostering care must, of course, be a plant of slow growth.

Mr. Hoffman's tables[97] show that in eight large Southern cities between 1891 and 1914, the first and last years covered, cancer mortality among whites increased by 73 per cent and that among negroes by 92 per cent. He has long given special attention to the statistics of negroes in the United States and notes that prior to the Civil War cancer among them was rare, that the present difference between the two races is less pronounced, but that the cancer mortality of negroes is still materially below that of whites.[98] These conclusions by one who accepts the opinion of qualified medical observers that "cancer is exceptionally rare among primitive peoples,"[99] are equally consistent with my own belief that much, if not all, of the difference is due to differences in the accuracy of diagnosis.

6. Has the death rate from cancer at the higher age periods increased more rapidly than earlier in life?

The Census Bureau has kindly furnished me with a table showing the cancer mortality in the registration states of 1900 classified by age and sex for the two years 1900 and 1910.[100] From these figures the following percentages of increase have been computed.

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[97] Op. cit., p. 474.

[98] Ibid., p. 15.

[99] Ibid., p. 147.

Per cent increase in the death rate from cancer by sex and age in the registration states of 1900 for the period 1900–1910

AGE	PER CENT INCREASE	
	Males	Females
25–34	4	16
35–44	17	13
45–54	28	17
55–64	44	24
65–74	31	33
75+	34	42

The figures are not perfectly regular and yet they do show clearly that in each sex the increase at the higher ages is more rapid than at the lower ages.

Similar evidence extending over a longer period of time is furnished by the Massachusetts records. The rates for a single year, when classified by sex and age, are often based on too few cases to be entirely trustworthy. The records cover the six census years 1860–1910, which, with due consideration of the sex differences, gives ten cases.[101] In one of the ten the most rapid increase was at ages over 80; in three it was at 70 to 79, in three it was at 60 to 69, and in only three was it at any period below 60, all three being at 30 to 39. This supports the previous conclusion that the increase of cancer mortality is greatest toward the end of a normal lifetime.

Similar rates for a series of decades are to be found in the series of decennial supplements to the Reports of the Registrar General and for 1901–1910 in his Seventy-third Annual Report for 1910. From them the following percentages of increase have been derived. As each rate speaks for a ten year period and for a large population, the results are more regular and more significant than those previously reached.

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[100] Appendix, table 31.

[101] Appendix, table 32.

Per cent of decennial increase in the death rate from cancer by sex and age in England and Wales

AGE	PER CENT OF DECENNIAL INCREASE			
	1861-1870 to 1871-1880	1871-1880 to 1881-1890	1881-1890 to 1891-1900	1891-1900 to 1901-1910
Males				
25–34	18	14	26	
35–44	17	25	29	13
45–54	32	42	30	25
55–64	33	45	38	28
65–74	74	44	42	34
75+	32	31	49	
Females				
25–34	9	0	2	
35–44	18	8	5	−1
45–54	16	16	14	5
55–64	20	22	22	11
65–74	26	28	29	22
75+	26	31	39	

The preceding figures all show that the increase in cancer mortality is greater at the higher ages. Incidentally the last series shows also that the increase of cancer among men is more rapid than among women and that during the last decades observed there was a slight fall in the cancer mortality of English women between thirty-five and forty-four years of age.

7. Is the increase in mortality from such forms of cancer as are difficult to diagnose more rapid than the increase in the forms easy to diagnose?

The classification of deaths from cancer made by Messrs. King and Newsholme into accessible and inaccessible cases suffers from one questionable assumption. It tacitly assumes that such a dual classification is possible. In fact cases of cancer fall into a series with those easy of diagnosis at one end and those very difficult of diagnosis at the other. One important element, perhaps the most important element, in determining the position of any specific case on that scale is the part of the body involved. A recent bulletin of the Federal Census Bureau[102] affords an objective test of this element by indicating what proportion of cases of each location were diagnosed by the reporting physician with reasonable certainty. It furnishes what students of the statistics of cancer have lacked hitherto, an objective basis for classifying deaths from this cause according to the certainty of the diagnosis and the part of the body first attacked. This classification has been derived from the judgment of the physicians who reported 52,420 deaths from this disease in 1914; and it is their judgment and the confidence they repose in it, rather than the judgment of a few men who are especially interested in the subject but report few, if any, deaths, which are of decisive importance. From these figures the following results have been derived (page 326).

These results furnish a test of the agreement beween the classification made by thousands of American physicians into cases of certain and uncertain diagnosis and the classification by Messrs. King and Newsholme into accessible and inaccessible cases. The English authorities included under accessible only cancers of the tongue, mamma, uterus and vagina. The following figures confirm this classification in every respect, but suggest that to these might well have been added cancers of other parts of the mouth than the tongue, of the skin, of the larynx, of the testes and of the rectum. But cancers of the skin, the mouth and the testes were not given separately in the Frankfort printed sources which they used, and they point out that, in their figures, cancers of the larynx and the rectum were so few or increased so little that a change in the classification of these forms of cancer would not affect the argument.

We may conclude that the dual classification of Messrs. King and Newsholme is justified by these results. A sharp line separates the accessible cases from the inaccessible and to the former class, in addition to those placed there by Messrs. King and Newsholme, belong cancers of the mouth, larynx, skin, rectum and testes. But the cases of inaccessible cancer may be ranged roughly in a series according to the reported uncertainty of the diagnosis from cancer of the ovary and fallopian tube at one extreme to cancer of the stomach, liver and gall bladder at the other. If the increase of cancer is largely due to improved diagnosis and the classification of cases according to the part of the body affected is correct and complete, then the rapidity of increase in the reported rate for inaccessible cancer of any particular internal organ and the period through which it persisted would probably vary with the difficulty of diagnosis. It

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[102] Mortality from Cancer and other Malignant Tumors in the Registration Area of the United States, 1914.

Deaths from cancer classified according to part of body affected, showing per cent with diagnosis uncertain, registration area of United States, 1914

PART OF BODY AFFECTED	NUMBER OF DEATHS EXCLUDING THOSE WITH DIAGNOSIS UNKNOWN	CASES WHERE DIAGNOSIS WAS UNCERTAIN	
		Number	Per cent
Breast	5,421	1	0
Skin	1,957	0	0
Jaw	851	1	0
Tongue	614	0	0
Lip	376	0	0
Mouth	230	0	0
Vagina and vulva	184	0	0
Testes	121	0	0
Uterus	7,468	8	0.1
Larynx	341	1	0.3
Rectum	2,164	14	0.6
Ovary and fallopian tube	366	56	15.3
Pancreas	567	219	38.6
Kidneys and suprarenals	434	171	39.4
Brain	98	39	39.8
Bladder	764	305	39.9
Peritoneum and mesentery	387	159	41.1
Bones (except jaw)	359	156	43.5
Prostate	625	314	50.2
Intestines	3,121	1,632	52.3
Lung and pleura	278	157	56.5
Pharynx	42	24	57.1
Esophagus	484	285	58.9
Liver and gall bladder	4,777	3,421	71.6
Stomach	9,681	6,973	72.0

may be wise in future to attempt an improvement of the method introduced by Messrs. King and Newsholme in conformity with this suggestion, but for the present the American material is entirely inadequate for such a study and that from other countries cannot be easily found or interpreted with confidence.

8. Is the rate of cancer mortality among men lower and its increase faster than among women?

The evidence upon which an affirmative answer to both branches of this question is based has already been reviewed in the examination of that part of Messrs. King and Newsholme's argument.

9. Does the increase of cancer mortality depend upon the initial rate, being high when that rate is small and diminishing or even disappearing when it approaches or reaches a certain high rate?

If the assumption which we are trying to test is correct, namely, that the true death rate from cancer varies only with age and that all other variations in the reported rates depend upon the accuracy of those rates and so upon the accuracy of diagnosis, then the increase would be likely to depend upon and vary with the initial rate. So long as the reported rate remained far below the true rate, the increase might be constant or might vary irregularly; but when the reported rate drew near the true rate and the opportunities for further improvements of diagnosis began to disappear, its increase would inevitably slacken. On the other hand, if the spread of the disease were actual, there would be no apparent reason why the disease should grow only towards or to some fixed limit. With the large amount of material recently brought together by the various international compilations, it has become possible to test this inference from our assumption on a scale hitherto quite out of the question.

We may test the theory first in its application to the 22 countries for which the cancer mortality is known. The death rate from cancer for each country in each year will be found in table 20 of the Appendix. From these rates quinquennial averages have been computed and the average increase in these per cents has been determined, the results being given in the following table.

RANGE OF QUINQUENNIAL RATES	NUMBER OF RATES INVOLVED	AVERAGE RATE OF INCREASE IN NEXT FIVE YEARS	
		Amount	Per cent
0–29	10	4.7	21.7
30–39	19	6.5	18.9
40–49	20	6.7	15.1
50–59	17	8.6	15.9
60–69	15	8.3	12.8
70–79	6	7.5	9.8
80–89	5	10.7	12.7
90–99	8	5.3	5.5
100+	5	4.0	3.5

With two exceptions the per cent of increase in the cancer mortality fell as the initial rate rose and after the initial rate reached 80 the average amount of increase also fell rapidly as the initial rate rose.

A similar line of argument has been applied to the larger and more trustworthy body of material compiled by Falkenburg and Hoffman for cities of at least 100,000 population. Each five year rate for each city has been obtained, these rates have been arranged in order of size, the increase (or decrease) between each five year rate and that for the following five years obtained and

RANGE OF QUINQUENNIAL RATES	NUMBER OF RATES INVOLVED	AVERAGE RATE OF INCREASE IN NEXT FIVE YEARS	
		Amount	Per cent
0– 9	11	1.0	19.1
10–19	6	8.4	59.4
20–29	11	8.2	31.7
30–39	32	6.3	17.9
40–49	63	7.2	16.0
50–59	76	7.5	13.6
60–69	83	7.1	11.1
70–79	74	9.6	12.9
80–89	44	6.4	7.5
90–99	46	4.1	4.3
100+	85	4.9	4.2

from these figures the average increase for each group within certain limits has been found.

Here, too, the average per cent of increase falls almost from the start as the initial rate rises and the amount of increase also falls rapidly after a certain rate, in this instance 70 per 100,000, is reached.

To supplement this information for the leading countries and cities of the world, I have turned to the returns of the annual volumes of American "Mortality Statistics" for the fifteen year period, 1900–1914. They embrace 49 American cities, each having at least 100,000 inhabitants in 1910, while the preceding tabulation included less than half as many American cities. In this case, instead of computing quinquennial averages, the rate in 1900 has been compared with that in 1914.

RANGE OF INITIAL RATES	NUMBER OF RATES INVOLVED	AVERAGE RATE OF INCREASE IN NEXT FOURTEEN YEARS	
		Amount	Per cent
30–39	3	42.0	116.0
40–49	5	31.3	68.7
50–59	10	31.6	58.6
60–69	18	28.4	44.6
70–79	6	21.7	29.1
80+	7	17.7	20.4

Here, too, it appears that the average per cent of increase slackens regularly and rapidly as the initial amount rises. The average amount of increase also falls regularly but the change does not appear until a certain rate, in this case 50 per 100,000, is reached.

One more effort to widen the inductive evidence has been made. In "Mortality Statistics, 1909," the death rates from cancer in each year of the preceding decade is reported for 142 cities which in 1910 had between 20,000 and 100,000 inhabitants. These cities are so small that it is unsafe to take the death rate of a single year as a standard. To avoid the difficulty, I have taken the average death rate from cancer during the five years 1900–1904 as a standard and have compared with that the average death rate in the same city for the years 1905–1909. In making this study a shorter period of time, five years instead of fourteen, is used and the results are correspondingly less important.

RANGE OF QUINQUENNIAL RATES	NUMBER OF RATES INVOLVED	AVERAGE RATE OF INCREASE IN NEXT FIVE YEARS	
		Amount	Per cent
0–39	9	12	35
40–59	54	11	22
60–79	50	9	15
80+	29	10	12

The preceding table shows that these smaller cities in general conform to the rule already illustrated in the rates for the foreign countries and larger foreign and American cities. In the death rate of the cities with a low initial rate the absolute increase somewhat exceeds that in cities with a high initial rate and the percentage of increase, which in my judgment is the fairer test, is much more rapid. The results from an extensive study of all these compilations of cancer statistics corroborate each other and constitute, I believe, strong evidence in support of the theory that the apparent increase in cancer mortality is not real.

Arguments from analogy are seldom of value in matters of this sort, but one analogy, that of appendicitis, seems to deserve mention. The opinions of physicians and surgeons regarding the actual increase of this disease diverge as widely as they do regarding the increase of cancer. Yet a confident belief in the increase of appendicitis is probably less general than the same belief regarding cancer, a larger proportion of medical men being ready to ascribe the increase of appendicitis to better diagnosis. Indeed many physicians are surprised to learn that in that part of the United States for which the facts are known the deaths reported from appendicitis are multiplying as fast as deaths from cancer. The following figures show the rates and the increase of each disease in the registration states of 1900 for each year between that date and the present.[103]

YEAR	DEATH RATE PER 100,000 POPULATION		RATIO OF RATE TO THAT OF 1900 = 100	
	Cancer	Appendicitis	Cancer	Appendicitis
1900	63.9	8.8	100	100
1901	65.8	8.8	103	100
1902	65.6	8.8	103	100
1903	69.0	9.6	108	109
1904	70.4	10.3	110	117
1905	72.4	10.2	113	116
1906	73.1	10.3	114	117
1907	75.7	10.2	118	116
1908	76.8	10.3	120	117
1909	79.4	10.8	124	123
1910	83.0	11.1	130	126
1911	83.9	11.3	131	128
1912	85.9	11.0	134	125
1913	88.7	11.6	139	132
1914	89.1	12.2	139	139
1915	91.8	12.3	144	140

A similar but more rapid spread of appendicitis in England is indicated by the reports. The mortality from it before 1901 is unknown. "The recorded death rate has increased from 38 per million in 1901 to 66 in 1910."[104] How much of this increase in either country is due to the better diagnosis of a disease which always attacks an inaccessible organ, I see no present means of determining.

SUMMARY OF RESULTS

The results of the preceding paper may be brought together under the following heads.

The reported mortality from cancer is increasing in almost every part of the world from which reports exist, but the real mortality, if it is increasing at all, is certainly not increasing with equal rapidity.

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[103] For complete figures see Appendix, table 33.

[104] England and Wales, 73rd Annual Report of the Registrar General (1910), p. lxxii.

To determine whether there is a real increase or to justify a denial of it, such parts of the reported increase as are not real must be measured and subtracted from the total, in order to ascertain whether there is a residuum not thus to be explained away.

For dealing with this problem, corrected, or standardized, death rates are likely to be misleading rather than helpful because the amount and rate of increase shown by them depend largely upon the standard which is used. This conclusion opens a way for using the data in the international compilations recently published, none of which are in the shape of corrected rates or can readily be converted into them.

Cancer mortality among men is lower but increases faster than among women. Both differences may be due to the fact that among men the organs chiefly attacked by cancer are inaccessible and among women the organs chiefly attacked are accessible. Hence the scope for improving the diagnosis of cancer among men was and is much wider.

The argument that the Frankfort corrected rates for accessible and inaccessible cancer, 1860–1890, indicate that the increase was confined to inaccessible cancer and, therefore, was probably due entirely to improved diagnosis is strengthened by compiling the Frankfort data 1890–1913 from manuscript sources in the registration office of that city. This new evidence, nearly doubling the period observed and trebling the material, strongly corroborates the statements based on the earlier Frankfort figures.

Notwithstanding the reinforcement of that conclusion by new evidence, the limited scope of the Frankfort material, the steady accumulation of other evidence pointing to a different conclusion and the doubt cast upon the usefulness of corrected death rates in attacking the present problem indicate that the question is still an open one.

A substitute for standardized rates as a way of measuring how much of the increase in the reported death rates from cancer is due to changes in the sex and age composition of a population during any period studied has been applied to five cases with the result that between one-sixteenth and fourteen-sixteenths and, on the average, one-third of the increase was found to be due to such changes. Evidently the part varies widely, but to assume that not less than one-tenth of the reported increase of cancer mortality is due to changes in sex and age composition during the period studied seems a conservative inference from the evidence.

The remainder, perhaps nine-tenths of the increase, must be explained by improved diagnosis, or accepted as actual.

The results of other intensive investigations add little to the evidence.

Statistics of the causes of death are obtained in different ways. In some places the corpse is examined by an inspector one of whose duties it is to certify the cause of death and get the diagnosis confirmed by the signature of the attending physician, in other places the attending physician certifies the cause directly and in still others whoever reports the death states the cause.

When the return comes from anyone but a physician, the cause of death is seldom reported as cancer.

Hence there is a uniform and close connection between the proportion of deaths certified by physicians and the proportion of deaths ascribed to cancer.

The reported cancer mortality is low in those Austrian provinces where medical certificates are few and higher where they are many. A similar relation is suggested in Europe when the countries are arranged in order of cancer mortality, Switzerland having the highest rate and Serbia the lowest rate.

This is of the first importance for the present problem, because in many countries the proportion of deaths certified by physicians is rapidly rising. Even when this is not the case, the proportion of ignorant and incompetent physicians is usually decreasing and this would tend to the same result.

At ages when the cancer mortality is highest the proportion of deaths certified by physicians to all deaths is much lower than in early middle life. Hence at those high ages there has been more room for a change from certification by a layman to certification by a physician and probably such changes have been made on a large scale.

The increase in cancer mortality is greatest at the upper ages, when the economic value of a life is least and diagnosis a few decades ago was probably most faulty.

The facilities for diagnosing cancer by microscopical examination of the diseased tissues, etc., are probably better in cities than in the country and better in hospitals than in general practice. Hence the disproportionate increase of urban population, the multiplication of hospital beds and, in many European countries, the increase of autopsies make for improved diagnosis of cancer.

The deaths ascribed to ill-defined or unknown causes in the American registration states of 1900 decreased between 1900 and 1915 to less than one-tenth of the initial number and the death rate still faster. If these deaths no longer ascribed to ill-defined or unknown causes were distributed over the other causes in a random way, the transfer from those causes to cancer accounted for about one-ninth of the increase in the reported mortality from cancer. This is probably an understatement of the proportion to be so explained. Similar changes have occurred in many other countries.

The deaths ascribed to tumor in the American registration states of 1900 decreased between 1900 and 1915 to a little over one-fourth and the rate to one-fourth of the initial figure. Where deaths were reported in 1900 only by enumerators, and so negligently, the proportion ascribed to tumor to all ascribed to tumor or cancer was seven times as great as within the registration area in the same year. The change from tumor to cancer in the registration states between 1900 and 1914 and in Massachusetts between 1855 and 1912 seems to account for one-twentieth of the increase in the cancer death rate.

The deaths ascribed to old age in the American registration states of 1900 decreased between 1900 and 1915 to a little more than two-fifths, and the rate to less than one-third, of the initial figure. The transfers from old age to cancer probably account for at least one-eighth of the increase of cancer mortality in those states, 1900 to 1915.

The measurable influences swelling the increase in the reported mortality from cancer above the true mortality, namely, the increasing proportion of elderly persons in the population, the transfers from ill-defined and unknown causes, from tumor and from old age to cancer seem to account in the American registration states for fully one-half of the reported increase, leaving another half to be otherwise explained or accepted as real.

Arguments contributing to explain away the remaining half of the increase in the reported cancer mortality either cannot yet be stated in the quantitative form preferred by statistics, or, if they can be thus stated, cannot be combined with the preceding by any process of addition.

If it should prove to be a general rule, as it apparently is true in Frankfort, that the increase is confined to mortality from inaccessible cancer, this would go far towards establishing the view that the whole increase was only apparent. But notwithstanding fragments of evidence from other districts, this form of argument is still restricted in the main to the Frankfort material.

In cities the cancer mortality is usually higher but increasing more slowly than in country districts and that notwithstanding the larger proportion of aged persons with high cancer mortality in the country. This may be connected with the larger proportion, greater accessibility and expertness of city physicians.

Cancer mortality among negroes in the United States is lower but increasing faster than among whites. It seems likely that this is due to marked but diminishing differences in correctness of diagnosis.

The increase in cancer mortality varies roughly with the size of the initial rate, tending to decrease, after a certain moderately high rate is reached, as the initial rate rises.

In England and the United States the increase in cancer mortality is parallel with the increase in mortality from appendicitis and both may be due entirely, as they certainly are in large degree, to the improvement of diagnosis.

The cumulative evidence that improvements in diagnosis and changes in age composition explain away more than half and perhaps all of the apparent increase in cancer mortality rebuts the presumption raised by the figures and makes it probable, although far from certain, that cancer mortality is not increasing.

APPENDIX

TABLE 1*

Death rate from cancer classified by sex for Connecticut, Maine, Massachusetts, New Hampshire and Rhode Island, by five year periods, 1896–1910

PERIOD	POPULATION	DEATHS FROM CANCER	DEATH RATE PER 100,000 POPULATION
		Male	
1896–1900	12,462,345	5,755	46.2
1901–1905	13,539,529	7,253	53.6
1906–1910	14,863,173	9,136	61.5
		Female	
1896–1900	12,839,093	11,592	90.3
1901–1905	13,891,724	14,016	100.9
1906–1910	15,102,303	16,741	110.9

* Data from Hoffman, pp. 456–469.

TABLE 2*

Death rate from cancer classified by sex, registration states as constituted in 1900,† for years 1900–1915

YEAR	POPULATION	DEATHS FROM CANCER	DEATH RATE PER 100,000 POPULATION
		Male	
1900	10,004,754	4,703	47.0
1901	10,222,010	4,982	48.7
1902	10,439,266	4,950	47.4
1903	10,656,522	5,393	50.6
1904	10,873,778	5,631	51.8
1905	11,091,034	5,881	53.0
1906	11,308,290	6,136	54.3
1907	11,525,546	6,454	56.0
1908	11,742,802	6,606	56.3
1909	11,960,058	7,115	59.5
1910	12,177,315	7,626	62.6
1911	12,394,571	7,962	64.2
1912	12,611,827	8,101	64.2
1913	12,829,083	8,617	67.2
1914	13,046,339	8,915	68.3
1915	13,263,595	9,589	72.3

* Figures from a manuscript table kindly furnished the writer by the Bureau of the Census.

† Maine, New Hampshire, Vermont, Massachusetts, Rhode Island, Connecticut, New York, New Jersey, District of Columbia, Indiana and Michigan.

TABLE 2—*Concluded*

YEAR	POPULATION	DEATHS FROM CANCER	DEATH RATE PER 100,000 POPULATION
		Female	
1900	9,990,458	8,066	80.7
1901	10,186,856	8,456	83.0
1902	10,383,254	8,703	83.8
1903	10,579,652	9,257	87.5
1904	10,776,051	9,616	89.2
1905	10,972,450	10,102	92.1
1906	11,168,849	10,290	92.1
1907	11,365,248	10,870	95.6
1908	11,561,647	11,290	97.7
1909	11,758,046	11,715	99.6
1910	11,954,444	12,398	103.7
1911	12,150,842	12,634	104.0
1912	12,347,240	13,350	108.1
1913	12,543,638	13,880	110.7
1914	12,740,037	14,052	110.3
1915	12,936,437	14,472	111.9

TABLE 3*

Death rate from cancer classified by sex, for fourteen American cities,† by five year periods, 1891–1910

PERIOD	POPULATION	DEATHS FROM CANCER	DEATH RATE PER 100,000 POPULATION
		Male	
1891–1895	12,805,336	5,059	39.4
1896–1900	16,461,766	7,543	45.8
1901–1905	20,382,334	10,631	52.1
1906–1910	23,045,672	14,245	61.8
		Female	
1891–1895	13,255,922	9,535	71.9
1896–1900	16,962,809	13,173	77.7
1901–1905	20,851,575	17,885	85.8
1906–1910	23,409,743	22,345	95.5

* Data from Hoffman, pp. 475 ff. The figures for New York City speak for Greater New York since 1898 and for Manhattan and Bronx Boroughs before that date. The cancer mortality of Brooklyn by sex 1891–1898 is unknown.

† Augusta, Ga., Boston, Cincinnati, Cleveland, Hartford, Nashville, New Haven, New York City, Philadelphia, Providence, Richmond, Springfield, Mass., St. Louis, Washington.

TABLE 4*

Death rate from cancer classified by sex, for England and Wales, Ireland, Norway, Bavaria, Italy, New South Wales, Victoria, South Australia, Queensland, Tasmania, New Zealand, Bermudas, and Jamaica, by five year periods, 1896–1910

PERIOD	POPULATION	DEATHS FROM CANCER	DEATH RATE PER 100,000 POPULATION
Male			
1896–1900	199,454,004	113,988	57.2
1901–1905	207,654,862	132,566	63.8
1906–1910	215,633,636	155,607	72.2
Female			
1896–1900	205,838,996	158,280	76.9
1901–1905	214,802,328	178,108	82.3
1906–1910	224,589,489	200,667	89.4

* Data from Hoffman, pp. 596, ff.

TABLE 5*

Death rate from cancer classified by sex, for certain foreign cities,† by five year periods, 1896–1910

PERIOD	POPULATION	DEATHS FROM CANCER	DEATH RATE PER 100,000 POPULATION
Male			
1896–1900	34,316,038	27,581	80.4
1901–1905	36,819,660	33,243	90.3
1906–1910	39,343,588	39,118	99.4
Female			
1896–1900	37,160,633	39,996	107.6
1901–1905	40,045,459	46,070	115.0
1906–1910	42,691,732	51,277	120.1

* Data from Hoffman, pp. 605, ff.

† London, Sheffield, Liverpool, Manchester, Cities of Denmark, Bremen, Berlin, Frankfort, Munich, Paris, Budapest, Sydney, Toronto, Buenos Aires.

TABLE 6*

Deaths from cancer in Frankfort-on-Main at all ages, classified by part of body affected

PART OF BODY AFFECTED	1860–1866		1867–1873		1874–1880		1881–1887		1888–1893†		1894–1899‡		1900–1904		1905–1909		1910–1913		TOTAL		
	Males	Females	Males	Females	Males.	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Both
Position undefined	33	29	18	30	42	20	58	55	31	32	26	29	26	38	42	74	42	63	318	370	688
Nervous system	5	3	8	5	4	1	10	10	2	4	3	3	4	6		1	2	2	38	35	73
Heart		1													3				3	1	4
Respiratory organs	2	3	2	1	8	4	8	3	8	12	20	11	29	13	39	36	31	23	147	106	253
Tongue	4		4		1	4	7	1	10	1	10	2	8	1	12		13	4	69	13	82
Esophagus and pharynx	6	2	5	3	15	4	30	11	41	4	62	10	69	17	87	15	51	16	366	82	448
Stomach	36	55	46	58	80	73	104	121	110	108	136	147	212	196	227	263	261	248	1212	1269	2481
Intestines	19	17	18	29	38	37	22	57	54	45	73	48	91	93	135	136	137	93	587	555	1142
Intra and retro-peritoneal	5	5	6	16	9	18	9	20	11	22	12	36	8	31	11	31	6	25	77	204	281
Liver	23	27	36	40	51	57	67	72	33	73	50	106	64	108	88	127	68	128	480	738	1218
Pancreas and spleen		1	1	1	4	1	1	4	1	4	5	3	9	11	8	9	5	4	34	38	72
Kidneys	1	1	4	2	4	2	7	3	7	1	9	3	6	3	10	5	5	8	53	28	81
Prostate, urinary bladder and penis	7	1	10	4	7	1	14	5	9	5	24	17	33	9	30	11	34	20	168	73	241
Uterus		69		96		117		159		158		148		182		206		161		1296	1296
Ovaries		9		9		17		20		50		14		8		14		13		134	131
Mamma		43		42		60		41	1	63		55		72		83	1	101	2	560	562
Vagina		1		2		5		5		5		4		2		3		20		47	47
Bone	2		2		5	1	5	1	4	4	13	11	13	13	16	23	12	14	72	67	139
Total	143	267	160	338	268	422	312	588	322	571	443	617	572	803	708	1037	668	943	3626	5616	9242

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* Figures from 1860 to 1887, inclusive, derived from King and Newsholme, table XIII. Figures for later years derived from manuscript tables from the Frankfort Statistical Office.

† Omitting the year 1890, for which the figures were not available.

‡ Omitting the year 1896, for the above reason.

TABLE 7*

Deaths from cancer in Frankfort-on-Main over twenty years of age

AGE	1860–1866		1867–1873		1874–1880		1881–1887		1888–1893†	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
20–29	3	5	5	3	7	5	6	15	1	9
30–39	10	13	17	32	19	40	34	50	15	53
40–49	18	51	20	65	47	92	57	123	66	101
50–59	50	86	40	95	60	112	94	143	106	166
60–69	36	70	47	80	79	99	88	151	80	153
70–79	19	35	26	54	43	67	46	75	47	66
80+	4	4	3	5	5	5	5	20	6	17
Total.....	140	264	158	334	260	420	330	577	321	565

AGE	1894–1899‡		1900–1904		1905–1909		1910–1913		TOTAL		
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Both
20–29	8	5	9	13	12	12	11	9	62	76	138
30–39	26	48	41	59	25	55	41	70	228	420	648
40–49	68	130	73	130	88	179	99	160	536	1,031	1,567
50–59	138	188	148	238	208	284	145	246	989	1,558	2,547
60–69	123	175	189	215	255	298	238	260	1,135	1,501	2,636
70–79	62	84	90	121	95	166	118	158	546	826	1,372
80+	10	14	17	25	15	34	7	32	72	156	228
Total	435	644	567	801	698	1,028	659	935	3,568	5,568	9,136

* Figures from 1860 to 1887, inclusive, derived from King and Newsholme, table XIV. Figures for later years derived from manuscript tables from the Frankfort Statistical Office.

† Omitting the year 1890, for which the figures were not available.

‡ Omitting the year 1896, for which the figures were not available.

TABLE 8*

Deaths from cancer in Frankfort-on-Main over twenty years of age, classified into accessible cancer, inaccessible cancer and cancer in undefined position

AGE	1860–1866		1867–1873		1874–1880		1881–1887		1888–1893†		1894–1899‡		1900–1904		1905–1909		1910–1913		TOTAL		
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Both
										Accessible cancer											
20–29		1						4		1			1	5		4		4	1	19	20
30–39		6	1	19		21	1	26	1	30		20		29		27		30	3	208	211
40–49		36	2	28		53	4	63	7	55	4	53	1	59	5	70	5	78	28	495	523
50–59	6	40		51		51	1	62	4	71	7	71	5	87	6	96	7	69	36	598	634
60–69	3	25	6	30		46		34	2	53	3	48	2	58	5	72	6	71	27	437	464
70–79		7		14	1	14	1	13	1	15		15	2	22	2	27	4	33	11	160	171
80+	1			1		1		4		4		4		6		9	2	10	3	39	42
Total..	10	115	9	143	1	186	7	206	15	229	14	211	11	266	18	305	24	295	109	1956	2065
										Inaccessible cancer											
20–29	1	4	3	3	6	5	1	10		8	6	5	8	7	11	7	10	4	46	53	99
30–39	4	6	13	11	17	15	21	21	11	20	22	27	39	26	23	25	37	32	187	183	370
40–49	14	9	15	33	40	36	44	46	51	41	63	72	67	66	77	96	89	70	460	469	929
50–59	34	36	35	34	50	54	80	64	93	91	122	108	141	146	191	167	134	164	880	864	1744
60–69	27	39	38	44	71	50	78	104	75	94	118	117	182	151	242	212	224	175	1055	986	2041
70–79	16	23	25	36	32	50	42	59	43	45	59	67	84	93	89	133	107	120	497	626	1123
80+	1	4	3	3	4	4	3	14	6	11	10	10	15	17	15	24	4	22	61	109	170
Total..	97	121	132	164	220	214	269	318	279	310	400	406	536	506	648	664	605	587	3186	3290	6476
										Cancer, position undefined											
20–29	2		2		1		5	1	1		2			1	1	1	1	1	15	4	19
30–39	6	1	3	2	2	4	12	3	3	3	4	1	2	4	2	3	4	8	38	29	67
40–49	4	6	3	4	7	3	9	14	8	5	1	5	5	5	6	13	5	12	48	67	115
50–59	10	10	5	10	10	7	13	17	9	4	9	9	2	5	11	21	4	13	73	96	169
60–69	6	6	3	6	8	3	10	13	3	6	2	10	5	6	8	14	8	14	53	78	131
70–79	3	5	1	4	10	3	3	3	3	6	3	2	4	6	4	6	7	5	38	40	78
80+	2			1	1		2	2		2			2	2		1	1		8	8	16
Total..	33	28	17	27	39	20	54	53	27	26	21	27	20	29	32	59	30	53	273	322	595

* Figures from 1860 to 1887, inclusive, derived from King and Newsholme, table XIV continued. Figures for later years derived from manuscript tables from the Frankfort Statistical Office.

† Omitting the year 1890, for which the figures were not available.

‡ Omitting the year 1896, for the same reason.

TABLE 9*

Population at Risk in Frankfort-on-Main

AGE	1860–1866	1867–1873	1874–1880	1881–1887	1888–1893†	1894–1899‡	1900–1904	1905–1909	1910–1913
Males									
20–29	90,404	76,174	90,222	96,183	97,888	134,459	173,683	192,245	169,211
30–39	42,294	49,166	69,410	87,654	70,964	93,702	124,430	154,841	158,092
40–49	29,201	30,964	40,993	57,471	51,794	65,878	77,958	93,107	97,586
50–59	18,924	30,036	23,860	30,224	29,154	41,070	51,297	57,152	53,499
60–69	10,850	11,556	12,918	16,158	13,586	18,124	24,008	29,540	29,912
70–79	3,700	4,228	5,116	6,292	5,109	6,506	7,898	9,240	9,984
80+	617	750	919	1,137	842	1,091	1,319	1,454	1,381
Total.	195,990	192,874	243,438	295,119	269,337	360,830	460,593	537,579	519,665
Females									
20–29	74,536	85,365	108,009	133,871	119,057	157,951	193,103	213,162	192,782
30–39	40,880	50,361	70,137	91,459	76,921	99,789	126,516	154,969	156,157
40–49	27,260	31,019	41,705	60,116	54,239	69,218	82,207	97,156	98,545
50–59	19,645	21,320	26,813	34,575	33,308	46,071	56,964	64,094	61,067
60–69	12,172	13,881	16,524	21,439	18,143	24,286	31,934	38,166	37,998
70–79	4,272	5,288	7,008	8,957	7,774	9,975	11,951	14,077	14,508
80+	1,110	944	1,077	1,904	1,632	1,879	2,296	2,630	2,490
Total.	179,875	208,178	271,273	352,321	311,074	409,169	504,971	581,254	563,547

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* Figures from 1860 to 1887, inclusive, derived from King and Newsholme, table XV. Figures for later years derived from German census report.

† Omitting the year 1890, for which the figures were not available.

‡ Omitting the year 1896, for which the figures were not available.

TABLE 10*

Frankfort. Annual deaths from cancer in 1,000,000 living, at least twenty-five years of age. Population distributed in age groups according to English Life Table No. 3—Persons

PERIOD	ACCESSIBLE	INACCESSIBLE	POSITION UNDEFINED	TOTAL
		Males		
1860–1866	126	1,118	359	1,603
1867–1873	88	1,421	137	1,646
1874–1880	14	1,913	363	2,290
1881–1887	35	1,865	305	2,205
1888–1893†	91	2,289	171	2,551
1894–1899‡	66	2,489	104	2,659
1900–1904	51	2,742	118	2,911
1905–1909	68	2,791	119	2,981
1910–1913	119	2,522	130	2,771
		Females		
1860–1866	1,081	1,323	293	2,697
1867–1873	1,214	1,540	254	3,008
1874–1880	1,220	1,588	131	2,939
1881–1887	981	1,820	272	3,073
1888–1893†	1,275	1,959	174	3,408
1894–1899‡	903	1,936	122	2,961
1900–1904	914	2,013	115	3,042
1905–1909	925	2,311	180	3,416
1910–1913	913	2,057	152	3,122

* Figures from 1860 to 1887, inclusive, derived from King and Newsholme, table XVI. Figures for later years derived from manuscript tables from the Frankfort Statistical Office.

† Omitting the year 1890, for which the figures were not available.

‡ Omitting the year 1896, for which the figures were not available.

TABLE 11

Standard million, England and Wales, as shown by English Life Table No. 3 and by Census of 1881

AGE PERIOD	ENGLISH LIFE TABLE NO. 3	CENSUS OF 1881
	Males	
Under 5	49,630	67,669
5–9	42,710	60,389
10–14	41,210	53,985
15–19	40,150	48,828
20–24	38,700	42,825
25–29	36,980	37,238
30–34	35,190	32,890
35–39	33,300	29,095
40–44	31,250	25,493
45–49	28,990	21,640
50–54	26,430	18,140
55–59	23,470	15,550
60–64	20,070	12,268
65–69	16,110	8,946
70–74	11,670	6,064
75–79	7,230	3,541
80–84	3,590	1,503
85–89	1,320	448
90–94	330	101
95+	50	15
	488,380	486,628
	Females	
Under 5	51,000	67,882
5–9	44,320	60,783
10–14	42,740	53,826
15–19	41,570	49,239
20–24	39,990	46,810
25–29	38,140	40,465
30–34	36,210	35,455
35–39	34,190	31,190
40–44	32,100	27,439
45–49	29,920	23,660
50–54	27,630	20,236
55–59	25,010	17,309
60–64	21,790	13,935
65–69	17,910	10,507
70–74	13,400	7,301
75–79	8,700	4,431
80–84	4,590	2,000
85–89	1,820	670
90–94	500	197
95+	90	37
	511,620	513,372

TABLE 12

Standardized cancer death rates for Frankfort, based on English Life Table No. 3 and on English census of 1881

AGE	STANDARD MILLION*		CANCER DEATH RATE FRANKFORT 1860–66†	COMPUTED CANCER DEATHS	
	English Life Table No. 3	English census of 1881		English Life Table No. 3	English census of 1881
			Males		
Under 20	173,700	230,871	2.7	5	6
20–29	75,680	80,063	3.5	3	3
30–39	68,490	61,985	20.5	14	13
40–49	60,240	47,133	53.1	32	25
50–59	49,900	33,690	225.1	112	76
60–69	36,180	21,214	278.7	101	59
70–79	18,900	9,605	453.9	86	44
80+	5,290	2,067	514.8	27	11
Total.........	488,380	486,628		380	237
			Females		
Under 20	179,630	231,730	3.0	5	7
20–29	78,130	87,275	6.0	5	5
30–39	70,400	66,645	27.0	19	18
40–49	62,020	51,099	156.2	97	80
50–59	52,640	37,545	372.4	196	140
60–69	39,700	24,442	477.8	190	117
70–79	22,100	11,732	693.5	153	81
80+	7,000	2,904	332.3	23	10
Total	511,620	513,372		688	458
Both sexes.....	1,000,000	1,000,000		1,068	695

Estimated cancer death rate based on:

English Life Table No. 3.............................	107 per 100,000
English census of 1881	70 per 100,000

* See the preceding table.

† Computed, using census population of Frankfort, 1864, from Stat. d. deut. Reichs, Beiträge zur Statistik d. St. Frankfurt-am-Main, II (1866), pt. I, table 19, and deaths from King and Newsholme, p. 240, supplemented as to deaths under 20 from personal investigation.

TABLE 13

Standardized cancer death rates for Frankfort, 1888–89, based on English Life Table No. 3 and on English census of 1881

AGE	STANDARD MILLION*		CANCER DEATH RATE PER 100,000 POPULATION. FRANKFORT, 1888-89†	COMPUTED CANCER DEATHS	
	English Life Table No. 3	English census of 1881		English Life Table No. 3	English census of 1881
			Males		
Under 20	173,700	230,871			
20–29	75,680	80,063	2.7	2	2
30–39	68,490	61,985	14.8	10	9
40–49	60,240	47,133	132.6	80	62
50–59	49,900	33,690	362.0	180	122
60–69	36,180	21,214	466.2	169	99
70–79	18,900	9,605	821.4	155	79
80+	5,290	2,067	625.0	33	13
Total ..	488,380	486,628		629	386
			Females		
Under 20	179,630	231,730	3.0	5	7
20–29	78,130	87,275	6.7	5	6
30–39	70,400	66,645	68.6	48	46
40–49	62,020	51,099	200.0	124	102
50–59	52,640	37,545	493.4	260	185
60–69	39,700	24,442	887.1	352	217
70–79	22,100	11,732	815.8	180	96
80+	7,000	2,904	937.5	66	27
Total	511,620	513,372		1,040	686
Both sexes. ..	1,000,000	1,000,000		1,669	1,072

Estimated cancer death rate based on:

English Life Table No. 3	167 per 100,000 population
English census of 1881	107 per 100,000 population

* See table 11.

† The death rates from cancer 1888-89 were computed by using the population of Frankfort December 1, 1889 (estimated by arithmetical method from population of 1885 and 1890) and the average annual deaths from cancer 1888-89, derived from King and Newsholme, Table xiv (this appendix, Table 7).

TABLE 14*

Death rate from cancer in the registration states of 1900 in the years 1900–1910

YEAR	POPULATION	DEATHS FROM CANCER	DEATH RATE PER 100,000 POPULATION
1900	19,995,212	12,769	63.9
1901	20,408,866	13,438	65.8
1902	20,822,520	13,653	65.6
1903	21,236,174	14,650	69.0
1904	21,649,829	15,247	70.4
1905	22,063,484	15,983	72.4
1906	22,477,139	16,426	73.1
1907	22,890,794	17,324	75.7
1908	23,304,449	17,896	76.8
1909	23,718,104	18,830	79.4
1910	24,131,759	20,024	83.0

Increase per 100,000 population, 1900–1910 .... 19.1
Per cent increase, 1900–1910 .... 29.9

* Data kindly furnished the writer in manuscript by the Bureau of the Census.

TABLE 15

*Standardized cancer death rate 1910 in registration states as constituted in 1900**

AGE PERIOD	MALES			FEMALES		
	Population 1900	Cancer death rate per 100,000 population 1910	Estimated deaths 1910	Population 1900	Cancer death rate per 100,000 population 1910	Estimated deaths 1910
Under 5	1,048,490	4.1	43	1,031,197	2.8	29
5–9	1,001,754	1.5	15	988,707	1.2	12
10–14	915,572	1.8	16	909,552	1.4	13
15–19	885,871	2.9	26	926,057	3.5	32
20–24	920,072	4.9	45	993,194	4.1	41
25–34	1,751,535	9.5	166	1,730,952	21.9	379
35–44	1,408,030	33.0	465	1,325,608	88.9	1,178
45–54	966,630	106.7	1,031	928,077	230.7	2,141
55–64	620,054	272.0	1,686	632,720	411.3	2,602
65–74	343,391	493.6	1,695	362,034	616.2	2,231
75+	143,355	693.7	994	162,360	867.8	1,409
Total........	10,004,754		6,182	9,990,458		10,067

Total population 19,995,212
Estimated deaths 16,249
Standardized death rate 81.3

* Population 1900 and cancer death rates 1910 kindly furnished the writer by the Bureau of the Census.

TABLE 16

Standardized cancer death rate, England and Wales, 1911

AGE PERIOD	MALES			FEMALES		
	Population* 1901	Cancer death rate per 100,000 population† 1911	Estimated deaths 1911	Population* 1901	Cancer death rate per 100,000 population† 1911	Estimated deaths 1911
Under 5	1,855,361	3.3	61	1,861,347	2.6	48
5–9	1,738,993	2.8	49	1,748,298	1.8	31
10–14	1,670,970	2.2	37	1,670,770	1.3	22
15–19	1,607,522	3.6	58	1,638,621	2.7	44
20–24	1,472,644	6.3	93	1,648,278	4.4	73
25–29	1,328,288	8.5	113	1,496,221	8.0	120
30–34	1,157,666	14.0	162	1,273,665	25.0	318
35–39	1,034,459	27.4	283	1,110,924	56.8	631
40–44	897,484	58.8	528	953,138	113.3	1,080
45–49	759,955	127.2	967	813,233	191.8	1,559
50–54	636,254	224.3	1,427	692,749	264.1	1,829
55–59	497,498	350.0	1,741	555,079	389.4	2,161
60–64	410,447	523.7	2,150	480,226	507.2	2,436
65–69	282,403	695.6	1,964	347,270	622.6	2,162
70–74	195,465	868.4	1,697	250,868	808.1	2,027
75–79	113,096	947.0	1,071	151,384	889.0	1,346
80–84	52,137	851.0	444	76,631	863.8	662
85+	17,971	738.9	133	30,528	808.4	247
Total	15,728,613		12,978	16,799,230		16,796

Total population	32,527,843
Estimated deaths	29,774
Standardized death rate	91.5

* From Census of England and Wales, 1901, Summary Tables, p. 139.

† Computed, using population for 1911 from Census of England and Wales, 1911, vii, pp. 1, 2; and deaths for 1911 from 74th Ann. Rep. of Registrar-General (1911), p. 201.

TABLE 17

Standardized cancer death rate of England and Wales, 1891–1900

AGE PERIOD	MALES			FEMALES		
	Standard million 1881–1890	Cancer death rate per 100,000 population 1891–1900	Estimated deaths 1891–1900	Standard million 1881–1890	Cancer death rate per 100,000 population 1891–1900	Estimated deaths 1891–1900
Under 5	64,122	3.3	2	64,557	2.8	2
5– 9	59,333	1.8	1	59,673	1.4	1
10–14	54,806	1.9	1	54,765	1.4	1
15–19	49,720	3.2	2	50,287	2.7	1
20–24	42,922	5.1	2	47,564	3.9	2
25–34	71,131	9.9	7	77,499	17.5	14
35–44	55,095	38.4	21	58,944	89.1	53
45–54	40,472	130.0	53	44,478	232.3	103
55–64	27,151	316.0	86	30,893	409.9	127
65–74	15,184	532.5	81	18,326	582.9	107
75+	5,591	582.4	33	7,487	637.7	48
Total . . .	485,527		289	514,473		459

Total estimated deaths per 1,000,000 748

Standardized death rate per 100,000. . . . . 74.8

* Standard million from Supp. to 65th Ann. Rep. of Registrar-General (1891–1900), p. xi; average annual deaths per million, *Ibid.*, p. cxcv.

TABLE 18

Standardized cancer death rate, New York City, 1910

AGE PERIOD	MALES			FEMALES		
	Population 1900	Cancer death rate per 100,000 population* 1910	Estimated deaths 1910	Population 1900	Cancer death rate per 100,000 population* 1910	Estimated deaths 1910
Under 5	199,683	7.0	14	197,604	2.8	6
5– 9	177,591	0.9	2	177,156	2.3	4
10–14	149,906	1.4	2	151,358	1.4	2
15–19	140,670	4.1	6	162,081	3.3	5
20–24	161,988	9.9	16	192,853	2.8	5
25–34	343,178	12.4	43	338,175	26.4	89
35–44	261,095	44.3	116	232,950	107.9	251
45–54	146,495	172.9	253	141,837	281.4	399
55–64	78,692	398.4	314	82,591	497.9	411
65–74	32,915	733.2	241	39,314	665.0	261
75+	9,799	681.4	67	13,835	884.6	122
Total . . .	1,702,012		1,074	1,729,754		1,555

Total population 3,431,766

Estimated deaths 2,629

Standardized death rate 76.6

* Death rates kindly furnished in manuscript by Bureau of the Census.

TABLE 19

Standardized cancer death rate, London, 1911*

AGE PERIOD	MALES			FEMALES		
	Population 1901†	Cancer death rate per 100,000 population‡ 1911	Estimated deaths 1911	Population 1901†	Cancer death rate per 100,000 population‡ 1911	Estimated deaths 1911
Under 15	675,970	3.5	24	681,904	2.3	16
15–24	426,271	5.5	23	493,478	3.8	19
25–34	371,418	11.8	44	437,763	16.0	70
35–39	150,706	30.4	46	168,882	60.2	102
40–44	129,992	64.0	83	141,486	128.0	181
45–49	107,320	164.9	177	119,010	170.5	203
50–54	87,913	279.5	246	99,284	268.1	266
55–59	65,784	439.3	289	75,821	392.5	298
60–64	52,932	661.3	352	65,153	508.2	331
65–69	33,341	855.0	285	45,125	591.9	267
70–74	21,662	1,130.3	245	32,647	764.7	250
75–79	11,816	1,104.1	130	19,612	942.7	185
80–84	5,218	993.6	52	10,200	963.6	98
85+	1,742	624.2	11	4,091	997.3	41
Total	2,142,085		2,007	2,394,456		2,327

Total population	4,536,541
Estimated deaths	4,334
Standardized death rate	95.5

* Administrative, or Registration, County.

† From Census of England and Wales, 1911, Summary Tables, pp. 148–149.

‡ From 74th Ann. Rep. of Registrar-General (1901), p. lxxvi.

TABLE 20*

Death rates from cancer in various European countries and the Australian colonies

YEAR	DEATH RATE FROM CANCER PER 100,000 POPULATION	YEAR	DEATH RATE FROM CANCER PER 100,000 POPULATION	YEAR	DEATH RATE FROM CANCER PER 100,000 POPULATION	YEAR	DEATH RATE FROM CANCER PER 100,000 POPULATION
			England and Wales†				
1858	32.9	1872	43.1	1886	59.0	1900	83.9
1859	33.7	1873	44.4	1887	61.5	1901	84.3
1860	34.1	1874	46.1	1888	62.2	1902	84.6
1861	36.0	1875	47.1	1889	65.6	1903	87.4
1862	36.1	1876	47.3	1890	67.6	1904	88.2
1863	36.1	1877	48.8	1891	69.2	1905	88.9
1864	38.6	1878	50.3	1892	69.2	1906	92.2
1865	37.2	1879	50.1	1893	71.0	1907	91.5
1866	38.5	1880	51.4	1894	71.2	1908	93.3
1867	39.2	1881	52.0	1895	75.3	1909	96.1
1868	40.3	1882	53.4	1896	76.4	1910	96.7
1869	41.7	1883	54.9	1897	78.5		
1870	42.4	1884	56.5	1898	79.9		
1871	42.4	1885	57.2	1899	82.6		
			Scotland				
1855	29.2	1869	43.1	1883	53.6	1897	74.7
1856	30.3	1870	41.1	1884	55.1	1898	79.5
1857	31.9	1871	42.5	1885	56.3	1899	81.3
1858	31.8	1872	45.8	1886	59.5	1900	78.9
1859	33.0	1873	44.7	1887	60.6	1901	81.8
1860	33.2	1874	47.3	1888	62.1	1902	82.3
1861	38.0	1875	47.6	1889	66.5	1903	83.8
1862	37.2	1876	47.7	1890	60.7	1904	85.9
1863	37.2	1877	48.2	1891	67.0	1905	90.0
1864	41.2	1878	50.4	1892	66.6	1906	97.6
1865	39.5	1879	49.9	1893	68.3	1907	97.9
1866	41.3	1880	50.5	1894	70.3	1908	98.5
1867	39.7	1881	51.1	1895	71.1	1909	101.6
1868	40.5	1882	54.5	1896	70.8	1910	102.7
			Ireland				
1864	26.6	1876	32.2	1888	41.7	1900	60.8
1865	28.6	1877	35.4	1889	44.9	1901	65.1
1866	30.7	1878	36.2	1890	45.5	1902	64.5
1867	30.2	1879	33.9	1891	46.2	1903	69.0
1868	30.1	1880	34.1	1892	47.9	1904	69.4
1869	30.5	1881	37.1	1893	49.5	1905	74.9
1870	33.1	1882	36.9	1894	51.8	1906	79.2
1871	32.1	1883	39.7	1895	50.4	1907	76.2
1872	32.6	1884	39.1	1896	53.7	1908	75.7
1873	32.7	1885	39.0	1897	58.2	1909	80.0
1874	33.4	1886	41.4	1898	58.8	1910	83.7
1875	33.2	1887	42.6	1899	59.0		

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* Computed from figures for population and deaths in Stat. Intern. du Mouvement de la Population, Vols. I and II.

† Figures going back to 1838 may be found in the successive Annual Reports of the Registrar-General.

TABLE 20—*Continued*

YEAR	DEATH RATE FROM CANCER PER 100,000 POPULATION	YEAR	DEATH RATE FROM CANCER PER 100,000 POPULATION	YEAR	DEATH RATE FROM CANCER PER 100,000 POPULATION	YEAR	DEATH RATE FROM CANCER PER 100,000 POPULATION
Norway							
1870	27.1	1881	45.7	1891	61.3	1901	95.1
1871	27.3	1882	46.2	1892	63.7	1902	91.5
1872	27.0	1883	49.4	1893	69.7	1903	93.2
1873	34.5	1884	51.6	1894	71.4	1904	96.0
1874	31.9	1885	53.1	1895	71.2	1905	98.4
1875	34.6	1886	50.4	1896	81.1	1906	97.6
1876	34.4	1887	55.6	1897	85.4	1907	100.3
1877	37.1	1888	55.2	1898	84.2	1908	97.2
1878	37.6	1889	56.4	1899	89.1	1909	95.2
1879	41.8	1890	57.0	1900	91.3	1910	92.9
1880	41.4						
Austria							
1873	33.0	1883	44.4	1893	59.0	1902	74.2
1874	33.9	1884	45.7	1894	60.8	1903	73.7
1875	35.2	1885	47.6	1895	63.7	1904	74.9
1876	36.4	1886	48.3	1896	65.8	1905	76.2
1877	37.4	1887	47.8	1897	67.9	1906	77.9
1878	38.4	1888	49.3	1898	69.4	1907	77.3
1879	40.7	1889	51.6	1899	69.9	1908	78.0
1880	40.7	1890	52.6	1900	70.9	1909	78.7
1881	42.2	1891	54.3	1901	72.9	1910	77.9
1882	42.7	1892	57.0				
Switzerland							
1877	78.9	1886	111.5	1895	122.2	1903	129.7
1878	82.4	1887	110.4	1896	120.0	1904	128.6
1879	86.6	1888	113.8	1897	123.4	1905	127.4
1880	90.7	1889	111.7	1898	123.3	1906	129.1
1881	99.3	1890	111.8	1899	122.0	1907	122.5
1882	99.1	1891	114.4	1900	125.4	1908	128.0
1883	101.0	1892	119.8	1901	127.9	1909	126.7
1884	103.9	1893	116.0	1902	125.8	1910	123.5
1885	106.5	1894	116.2				
German Empire‡							
1892	61.0	1897	69.3	1902	75.0	1907	83.2
1893	63.0	1898	70.4	1903	77.2	1908	83.7
1894	64.5	1899	73.7	1904	79.8	1909	84.5
1895	65.7	1900	72.1	1905	80.9	1910	87.9
1896	68.1	1901	74.7	1906	81.3		

‡ Figures include about 94 per cent of the population. For details see notes in Stat. Intern. du Mouvement de la Population, I and II.

TABLE 20—*Continued*

YEAR	DEATH RATE FROM CANCER PER 100,000 POPULATION	YEAR	DEATH RATE FROM CANCER PER 100,000 POPULATION	YEAR	DEATH RATE FROM CANCER PER 100,000 POPULATION	YEAR	DEATH RATE FROM CANCER PER 100,000 POPULATION
Prussia							
1875	22.9	1884	35.3	1893	51.1	1902	61.9
1876	24.2	1885	35.8	1894	52.8	1903	65.2
1877	26.5	1886	38.3	1895	53.2	1904	68.6
1878	28.2	1887	38.1	1896	54.8	1905	69.4
1879	28.8	1888	40.9	1897	56.0	1906	70.4
1880	29.9	1889	43.5	1898	56.3	1907	73.4
1881	31.1	1890	43.3	1899	59.3	1908	73.6
1882	31.9	1891	44.7	1900	59.6	1909	74.8
1883	33.8	1892	49.6	1901	61.7	1910	78.5
Bavaria							
1888	79.4	1894	92.6	1900	99.3	1906	110.5
1889	81.6	1895	94.9	1901	102.9	1907	106.8
1890	81.0	1896	95.3	1902	103.4	1908	108.2
1891	88.7	1897	96.9	1903	107.4	1909	109.7
1892	85.0	1898	97.3	1904	110.2	1910	114.2
1893	90.0	1899	101.9	1905	108.9		
*Saxony**							
1892	85.7	1897	95.2	1902	97.0	1907	97.3
1893	85.7	1898	91.2	1903	91.0	1908	94.3
1894	89.1	1899	94.8	1904	88.2	1909	93.9
1895	89.5	1900	93.6	1905	91.6	1910	93.9
1896	93.2	1901	97.4	1906	93.8		
Wurtemberg							
1892	71.4	1897	80.0	1902	97.4	1907	102.8
1893	71.4	1898	95.0	1903	98.4	1908	102.9
1894	70.7	1899	94.0	1904	98.7	1909	105.5
1895	75.2	1900	94.0	1905	104.2	1910	107.3
1896	82.1	1901	94.4	1906	99.3		
Holland							
1875	47.9	1884	61.6	1893	80.8	1902	95.0
1876	48.5	1885	66.0	1894	81.0	1903	98.9
1877	50.2	1886	67.0	1895	85.4	1904	97.9
1878	51.1	1887	65.3	1896	88.5	1905	101.2
1879	52.5	1888	69.5	1897	90.4	1906	100.7
1880	54.4	1889	75.3	1898	93.0	1907	101.8
1881	57.6	1890	73.4	1899	96.0	1908	102.8
1882	58.4	1891	79.4	1900	91.8	1909	102.6
1883	58.0	1892	79.9	1901	93.7	1910	106.4

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* For figures running back to 1873 see Zeitschr. d. k. Säch. Stat. Landesamtes 1905, p. 263.

TABLE 20—*Continued*

YEAR	DEATH RATE FROM CANCER PER 100,000 POPULATION	YEAR	DEATH RATE FROM CANCER PER 100,000 POPULATION	YEAR	DEATH RATE FROM CANCER PER 100,000 POPULATION	YEAR	DEATH RATE FROM CANCER PER 100,000 POPULATION
			Spain				
1900	39.3	1903	44.1	1906	47.6	1909	51.0
1901	42.4	1904	46.6	1907	47.5	1910	52.3
1902	43.3	1905	45.8	1908	51.4		
			Italy				
1887	42.7	1893	42.9	1899	51.9	1905	58.3
1888	42.3	1894	41.5	1900	52.2	1906	62.0
1889	43.0	1895	48.2	1901	52.7	1907	61.7
1890	42.7	1896	49.1	1902	53.9	1908	64.5
1891	43.0	1897	50.3	1903	53.8	1909	64.2
1892	42.6	1898	51.1	1904	57.1	1910	65.6
			Serbia				
1895	5.5	1899	9.5	1903	9.1	1907	13.3
1896	6.6	1900	9.5	1904	10.3	1908	12.6
1897	7.0	1901	9.1	1905	10.4	1909	13.1
1898	7.4	1902	9.6	1906	10.6	1910	12.8
			New South Wales				
1875	30.9	1884	26.4	1893	40.7	1902	62.3
1876	27.5	1885	28.8	1894	42.1	1903	65.4
1877	26.6	1886	31.4	1895	44.4	1904	66.0
1878	29.0	1887	35.2	1896	48.9	1905	65.3
1879	24.7	1888	39.0	1897	53.3	1906	69.0
1880	32.8	1889	36.9	1898	54.4	1907	70.8
1881	28.2	1890	35.6	1899	57.1	1908	67.5
1882	26.9	1891	45.2	1900	56.5	1909	72.5
1883	25.7	1892	43.3	1901	61.7	1910	71.7
			Victoria				
1864	21.7	1876	38.0	1888	49.4	1900	68.5
1865	24.7	1877	40.7	1889	57.7	1901	73.4
1866	18.4	1878	38.3	1890	56.0	1902	70.4
1867	18.6	1879	45.9	1891	60.9	1903	76.1
1868	27.6	1880	45.1	1892	58.8	1904	73.9
1869	26.8	1881	40.4	1893	62.6	1905	78.6
1870	30.0	1882	41.9	1894	63.1	1906	75.5
1871	26.2	1883	49.5	1895	64.2	1907	82.1
1872	30.0	1884	47.8	1896	66.7	1908	81.8
1873	32.6	1885	46.5	1897	65.5	1909	82.6
1874	34.5	1886	50.4	1898	73.1	1910	82.5
1875	39.1	1887	51.8	1899	71.0		

TABLE 20—*Concluded*

YEAR	DEATH RATE FROM CANCER PER 100,000 POPULATION	YEAR	DEATH RATE FROM CANCER PER 100,000 POPULATION	YEAR	DEATH RATE FROM CANCER PER 100,000 POPULATION	YEAR	DEATH RATE FROM CANCER PER 100,000 POPULATION
Queensland							
1880	23.0	1888	23.3	1896	39.3	1904	57.2
1881	29.4	1889	32.7	1897	39.1	1905	66.7
1882	22.7	1890	28.2	1898	46.5	1906	54.7
1883	26.1	1891	33.1	1899	47.8	1907	66.4
1884	31.4	1892	31.3	1900	47.7	1908	61.0
1885	16.7	1893	28.3	1901	55.0	1909	60.7
1886	27.6	1894	35.8	1902	55.0	1910	67.3
1887	22.8	1895	41.7	1903	49.1		
South Australia							
1880	28.1	1888	37.8	1896	52.5	1904	63.4
1881	32.6	1889	43.1	1897	51.8	1905	69.2
1882	31.0	1890	41.4	1898	51.1	1906	76.9
1883	29.2	1891	49.1	1899	57.0	1907	74.5
1884	35.9	1892	45.5	1900	59.8	1908	73.6
1885	32.8	1893	50.9	1901	60.5	1909	80.0
1886	34.1	1894	47.0	1902	75.0	1910	80.7
1887	36.0	1895	47.4	1903	73.5		
West Australia							
1895	27.2	1899	35.5	1903	41.6	1907	50.0
1896	25.2	1900	29.7	1904	44.3	1908	54.3
1897	34.0	1901	44.4	1905	50.8	1909	68.0
1898	33.3	1902	41.3	1906	59.2	1910	49.5
Tasmania							
1895	48.7	1899	53.2	1903	56.2	1907	62.9
1896	60.1	1900	53.8	1904	52.0	1908	67.6
1897	50.0	1901	54.6	1905	54.2	1909	67.4
1898	59.3	1902	61.9	1906	51.9	1910	66.5
New Zealand							
1880	28.1	1888	43.5	1896	55.1	1904	67.6
1881	27.2	1889	42.5	1897	54.7	1905	65.1
1882	28.9	1890	47.5	1898	64.0	1906	69.5
1883	29.9	1891	46.8	1899	62.4	1907	73.5
1884	34.5	1892	47.8	1900	56.3	1908	70.8
1885	31.1	1893	50.2	1901	66.2	1909	74.1
1886	36.8	1894	60.1	1902	67.2	1910	76.1
1887	39.9	1895	55.4	1903	71.0		

TABLE 21*

Proportion of total deaths occurring in hospitals, New York City, 1898–1915

YEAR	TOTAL DEATHS	DEATHS IN HOSPITALS	PER CENT OCCURRING IN HOSPITALS
1898	66,294	10,799	16.3
1899	65,343	11,749	18.0
1900	70,872	12,609	17.8
1901	70,720	13,343	18.9
1902	68,112	12,613	18.5
1903	67,864	14,695	21.6
1904	78,060	17,138	22.0
1905	73,714	18,103	24.6
1906	76,203	19,163	25.1
1907	79,205	21,444	27.1
1908	73,072	20,684	28.3
1909	74,105	21,451	28.9
1910	76,742	22,631	29.5
1911	75,423	23,466	31.1
1912	73,008	22,198	30.4
1913	73,902	22,788	30.8
1914	74,803	23,823	31.8
1915	76,193	25,095	32.9

* Figures kindly furnished the writer by the Bureau of Records, New York City. Hospitals, as here used, include only those institutions "which individuals enter direct from their homes," and not those "of which decedents were residents at the time of death."

TABLE 22*

Proportion of deaths in hospitals to total deaths, former city of New York, 1889–1897

YEAR	TOTAL DEATHS	DEATHS IN HOSPITALS	PER CENT OF DEATHS OCCURRING IN HOSPITALS
1889	39,583	6,102	15.4
1890	40,103	6,310	15.7
1891	43,659	6,948	15.9
1892	44,329	7,681	17.3
1893	44,486	8,306	18.7
1894	41,175	7,742	18.8
1895	43,420	8,091	18.6
1896	41,622	8,530	20.4
1897	38,857	8,534	22.0

* Data derived from the successive Annual Reports of the New York City Department of Health.

TABLE 23*

Hospital Beds to 100,000 Population, German Empire, 1877-1910

YEAR	HOSPITAL BEDS PER 100,000 POPULATION
1877	166
1879	175
1882	182
1885	201
1888	224
1891	246
1894	266
1897	273
1900	295
1901	310
1902	325
1903	335
1904	345
1905	356
1906	364
1907	376
1908	382
1909	391
1910	400

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* Computed from *Stat. Jahrb. für d. D. Reich*, xxxvi (1915), population p. 2; hospital statistics p. 446.

TABLE 24

Proportion of deaths from cancer and from tumor to deaths from both causes, United States, 1880–1914

YEAR	DEATHS FROM			PER CENT CANCER	PER CENT TUMOR
	Cancer and tumor	Cancer	Tumor		
United States (Entire Area)					
1880	14,849	13,068	1,781	88.0	12.0
1890	20,984	18,536	2,448	88.3	11.7
1900	32,902	29,475	3,427	89.6	10.4
*United States (Registration Area)**					
1900	19,962	19,381	581	97.1	2.9
1901	20,676	20,171	505	97.5	2.5
1902	21,306	20,847	459	97.8	2.2
1903	22,856	22,325	531	97.7	2.3
1904	23,839	23,395	444	98.1	1.9
1900–1904	108,639	106,119	2,520	97.7	2.3
1905	24,831	24,330	501	98.0	2.0
1906	29,490	29,020	470	98.4	1.6
1907	31,022	30,514	508	98.4	1.6
1908	33,923	33,465	458	98.6	1.4
1909	38,020	37,562	458	98.8	1.2
1905–1909	157,286	154,891	2,395	98.5	1.5
1910	41,592	41,039	553	98.7	1.3
1911	44,479	44,024	455	99.0	1.0
1912	46,908	46,531	377	99.2	0.8
1913	50,225	49,928	297	99.4	0.6
1914	52,756	52,420	336	99.4	0.6
1910–1914	235,960	233,942	2,018	99.1	0.9

* See comments, pp. 309 and 310.

TABLE 25*

Death rates from cancer and from tumor in the registration states of 1900, for the years 1900–1915

YEAR	POPULATION	DEATHS FROM			DEATH RATE PER 100,000 POPULATION			PER CENT OF DEATHS FROM CANCER
		Cancer	Tumor	Both	Cancer	Tumor	Both	
1900	19,995,212	12,769	394	13,163	63.9	2.0	65.9	97.0
1901	20,408,866	13,438	332	13,770	65.8	1.6	67.4	97.6
1902	20,822,520	13,653	287	13,940	65.6	1.4	67.0	97.9
1903	21,236,174	14,650	355	15,005	69.0	1.7	70.7	97.6
1904	21,649,829	15,247	294	15,541	70.4	1.4	71.8	98.1
1905	22,063,484	15,983	318	16,301	72.4	1.4	73.8	98.1
1906	22,477,139	16,426	284	16,710	73.1	1.3	74.4	98.3
1907	22,890,794	17,324	278	17,602	75.7	1.2	76.9	98.5
1908	23,304,449	17,896	229	18,125	76.8	1.0	77.8	98.7
1909	23,718,104	18,830	227	19,057	79.4	1.0	80.4	98.8
1910	24,131,759	20,024	294	20,318	83.0	1.2	84.2	98.6
1911	24,545,413	20,596	203	20,799	83.9	0.8	84.7	99.1
1912	24,959,067	21,451	169	21,620	85.9	0.7	86.6	99.2
1913	25,372,721	22,497	119	22,616	88.7	0.5	89.2	99.4
1914	25,786,376	22,967	144	23,111	89.1	0.6	89.7	99.3
1915	26,200,032	24,061	138	24,199	91.8	0.5	92.3	99.4

* Population, deaths and death rates from cancer kindly furnished the writer in manuscript by the Bureau of the Census; figures for tumor compiled from successive volumes of "Mortality Statistics."

TABLE 26*

Death rates from cancer and from tumor, Massachusetts, by quinquennial periods, 1853–1912

PERIOD	MID-PERIOD CENSUS POPULATION	AVERAGE ANNUAL NUMBER OF DEATHS FROM			DEATH RATES PER 100,000 POPULATION			PER CENT OF DEATHS FROM CANCER
		Cancer	Tumor	Both	Cancer	Tumor	Both	
1853–1857	1,132,369	219	60	279	19.3	5.3	24.6	78.5
1858–1862	1,231,066	317	61	378	25.8	4.9	30.7	83.9
1863–1867	1,267,031	376	67	443	29.7	5.3	35.0	84.9
1868–1872	1,457,351	509	84	593	34.9	5.8	40.7	85.8
1873–1877	1,651,912	618	90	708	37.4	5.4	42.8	87.3
1878–1882	1,783,085	907	68	975	50.8	3.8	54.6	93.0
1883–1887	1,942,141	1,090	64	1,154	56.1	3.3	59.4	94.5
1888–1892	2,238,943	1,357	70	1,427	60.6	3.1	63.7	95.1
1893–1897	2,500,183	1,677	95	1,772	67.1	3.8	70.9	94.6
1898–1902	2,805,346	1,993	85	2,078	71.0	3.0	74.0	96.0
1903–1907	3,003,680	2,502	76	2,578	83.3	2.5	85.8	97.1
1908–1912	3,366,416	3,039	42	3,081	90.2	1.2	91.4	98.6

* Population from Federal and state censuses; deaths from cancer and tumor from successive state Registration Reports.

TABLE 27*

Death rates from old age in the registration states of 1900, for the years 1900–1915

YEAR	POPULATION	DEATHS FROM OLD AGE	DEATH RATES FROM OLD AGE PER 100,000 POPULATION
1900	19,995,212	10,015	50.1
1901	20,408,866	9,771	47.9
1902	20,822,520	9,309	44.7
1903	21,236,174	8,604	40.5
1904	21,649,829	8,702	40.2
1905	22,063,484	8,251	37.4
1906	22,477,139	8,432	37.5
1907	22,890,794	7,614	33.3
1908	23,304,449	6,943	29.8
1909	23,718,104	6,472	27.3
1910	24,131,759	6,321	26.2
1911	24,545,413	6,070	24.7
1912	24,959,067	6,426	25.8
1913	25,372,721	5,795	22.8
1914	25,786,376	4,901	19.0
1915	26,200,032	4,380	16.7

* Population figures from manuscript table kindly furnished by the Bureau of the Census; deaths from old age from the successive volumes of Mortality Statistics.

TABLE 28*

Death rate from cancer in cities and rural districts of the registration states of 1900, for years 1900–1915

YEAR	POPULATION		DEATHS FROM CANCER		DEATH RATE PER 100,000 POPULATION	
	Cities	Rural districts	Cities	Rural districts	Cities	Rural districts
1900	10,690,666	9,304,546	7,060	5,709	66.0	61.4
1901	11,105,137	9,303,729	7,717	5,721	69.5	61.5
1902	11,519,608	9,302,912	7,844	5,809	68.1	62.4
1903	11,934,080	9,302,094	8,498	6,152	71.2	66.1
1904	12,348,553	9,301,276	8,892	6,355	72.0	68.3
1905	12,763,026	9,300,458	9,323	6,660	73.0	71.6
1906	13,177,499	9,299,640	10,065	6,361	76.4	68.4
1907	13,591,972	9,298,822	10,544	6,780	77.6	72.9
1908	14,006,445	9,298,004	10,809	7,087	77.2	76.2
1909	14,420,918	9,297,186	11,530	7,300	80.0	78.5
1910	14,838,879	9,292,880	12,302	7,722	82.9	83.1
1911	15,202,813	9,342,600	12,760	7,836	83.9	83.9
1912	15,565,218	9,393,849	13,437	8,014	86.3	85.3
1913	15,927,982	9,444,739	14,042	8,455	88.2	89.5
1914	16,294,421	9,491,955	14,546	8,421	89.3	88.7
1915	16,662,676	9,537,356	15,208	8,853	91.3	92.8

* Data kindly furnished the writer in manuscript by the Bureau of the Census.

TABLE 29*

Death rates from cancer in certain European countries classified by city and rest of country

YEAR	POPULATION		DEATHS FROM CANCER		DEATH RATES FROM CANCER PER 100.000 POPULATION	
	Urban	Rest of country	Urban	Rest of country	Urban	Rest of country
			England and Wales†			
1881	5,058,823	20,987,319	2,898	10,644	57.3	50.7
1891	5,546,621	23,539,198	4,217	15,900	76.0	67.5
1901	6,141,048	26,471,086	5,553	21,934	90.4	82.9
1909	6,222,293	29,205,379	6,687	27,366	107.5	93.7
			Scotland‡			
1881	740,890	3,001,674	424	1,490	57.2	49.6
1891	829,394	3,206,851	584	2,119	70.4	66.1
1901	1,092,515	3,386,550	864	2,798	79.1	82.6
1909	1,102,996	3,604,862	1,213	3,569	110.0	99.0
			Norway§			
1881	120,722	1,793,278	105	769	87.0	42.9
1891	153,887	1,842,113	147	1,077	95.5	58.5
1901	228,336	2,006,664	222	1,904	97.2	94.9
1909	239,704	2,098,296	239	1,987	99.7	94.7
			*Netherlands***			
1881	683,889	3,403,445	475	1,878	69.5	55.2
1891	890,776	3,702,379	743	2,905	83.4	78.5
1901	1,193,689	4,023,554	1,104	3,790	92.5	94.2
1909	1,366,862	4,453,613	1,385	4,627	101.3	103.9
			Austria††			
1881	976,535	21,232,465	1,162	8,202	119.0	38.6
1891	1,683,096	22,312,904	2,018	11,009	119.9	49.3
1901	2,065,344	24,113,412	2,462	16,692	119.2	69.2
1908	2,416,104	25,427,421	3,207	18,973	132.7	74.6

* Data from Hoffman, supplemented by material from Falkenburg and March.

† Cities are Birmingham, Bradford, Derby, Liverpool and London.

‡ Cities are Edinburgh and Glasgow.

§ City is Christiania.

** Cities are Amsterdam, the Hague, Rotterdam and Utrecht.

†† Cities are Prague and Vienna.

TABLE 30*

Death rates from cancer, classified by color of decedent, in the registration states of 1910, for the years 1910–1915

YEAR	POPULATION	DEATHS FROM CANCER	DEATH RATE FROM CANCER PER 100,000 POPULATION
		Total	
1910	47,807,766	36,364	76.1
1911	48,756,771	37,269	76.4
1912	49,595,220	39,279	79.2
1913	50,493,332	41,430	82.1
1914	51,455,677	42,706	83.0
1915	52,357,716	44,440	84.9
		White	
1910	46,425,660	35,638	76.8
1911	47,344,587	36,432	77.0
1912	48,153,883	38,424	79.8
1913	49,025,379	40,490	82.6
1914	49,941,006	41,716	83.5
1915	50,845,959	43,400	85.4
		Colored	
1910	1,382,106	726	52.6
1911	1,412,184	837	59.3
1912	1,441,337	855	59.3
1913	1,467,953	940	64.0
1914	1,514,671	990	65.4
1915	1,511,757	1,040	68.8

* Figures for 1910-1915 from a manuscript table kindly furnished the writer by the Bureau of the Census.

TABLE 31*

Death rates from cancer classified by sex and age, and rate of decennial increase, registration states of 1900, for 1900 and 1910

AGE	MALES			FEMALES		
	Death rate from cancer per 100,000 population		Rate of increase 1900–1910	Death rate from cancer per 100,000 population		Rate of increase 1900–1910
	1900	1910		1900	1910	
25–34	9.1	9.5	4	18.9	21.9	16
35–44	28.1	33.0	17	78.8	88.9	13
45–54	83.5	106.7	28	196.6	230.7	17
55–64	189.3	272.0	44	330.5	411.3	24
65–74	377.4	493.6	31	461.6	616.2	33
75+	516.9	693.7	34	609.1	867.8	42

* From a manuscript table kindly furnished the writer by the Bureau of the Census.

TABLE 32*

Death rates from cancer per 100,000 population classified by sex and age, Massachusetts, 1860–1910

AGE PERIOD	1860	1870	1880	1890	1900	1910
Males						
30–39	6.6	6.9	11.9	7.1	16.9	17.9
40–49	20.4	27.5	39.0	29.3	62.5	61.4
50–59	63.4	83.4	99.7	118.2	145.6	161.6
60–69	105.7	154.2	168.7	267.1	277.6	415.9
70–79	166.6	267.2	408.3	332.8	497.1	658.4
80+	349.7	354.8	491.1	331.7	655.4	955.1
Females						
30–39	17.1	23.0	46.3	45.5	38.1	48.5
40–49	79.5	84.8	116.0	138.2	149.2	160.5
50–59	145.2	139.7	219.4	261.8	284.5	317.9
60–69	162.0	216.9	282.7	387.6	451.4	558.5
70–79	202.5	254.8	371.9	527.3	555.2	817.7
80+	341.4	379.4	320.2	438.2	680.1	861.4

Per cent of decennial increase in death rate

AGE PERIOD	1860–1870	1870–1880	1880–1890	1890–1900	1900–1910
Males					
30–39	4.5	72.5	−40.3†	138.0	5.9
40–49	4.2	41.8	−24.9†	113.3	−1.8†
50–59	31.5	19.5	18.6	23.2	11.0
60–69	45.9	9.4	58.3	3.9	49.8
70–79	60.4	52.8	−18.5†	49.4	32.5
80+	1.5	38.4	−32.5†	97.6	45.7
Females					
30–39	34.5	101.3	−1.7†	−16.3†	27.3
40–49	6.7	36.8	19.1	8.0	7.6
50–59	−3.8†	57.1	19.3	8.7	11.7
60–69	33.9	30.3	37.1	16.5	23.7
70–79	25.8	46.0	41.8	5.3	47.3
80+	11.1	−15.6†	36.8	55.2	26.7

* Population figures used in computing these rates are those of the Federal censuses; deaths, those in the successive Massachusetts Registration Reports.

† Decrease.

TABLE 33*

Death rate from appendicitis in the registration states of 1900, for the years 1900–1915

YEAR	POPULATION	DEATHS FROM APPENDICITIS	DEATH RATE PER 100,000 POPULATION
1900	19,995,212	1,763	8.8
1901	20,408,866	1,794	8.8
1902	20,822,520	1,825	8.8
1903	21,236,174	2,031	9.6
1904	21,649,829	2,224	10.3
1905	22,063,484	2,256	10.2
1906	22,477,139	2,318	10.3
1907	22,890,794	2,326	10.2
1908	23,304,449	2,396	10.3
1909	23,718,104	2,550	10.8
1910	24,131,759	2,685	11.1
1911	24,545,413	2,783	11.3
1912	24,959,067	2,743	11.0
1913	25,372,721	2,940	11.6
1914	25,786,376	3,149	12.2
1915	26,200,032	3,221	12.3

* Data kindly furnished the writer in manuscript by the Bureau of the Census.

TUMORS OF THE KIDNEY IN RABBITS

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Jacob Wolff (1) has commented upon the infrequency of new growths in the rabbit, citing only twelve well defined examples, with four other cases referred to as cystadenomas of the mammary gland, general lymphosarcomatosis, and fungus haematoidis. Recently Bell and Henrici (2) have collected twenty-four additional cases, and have reported two renal tumors occurring in their own experience. Eliminating the four distinctly doubtful cases there are thus but thirty-eight new growths of the rabbit described in the literature. It has been suggested that a possible explanation for this infrequency lies in the fact that careful postmortem examinations are made only upon animals used in the laboratory, and that these animals seldom reach the age of maximum incidence for tumors.

Of the thirty-eight reported neoplasms of rabbits, four have occurred in the kidney. Three of these were very similar in their histological structure, and the fourth varied only in the presence of nonstriated muscle fibers in its capsule. The fifth renal tumor, about to be reported, is so nearly like those already recorded that a single description would be sufficient to cover all.

The present case occurred in a Belgian hare which died after inoculation with the virus of rabies. The animal had recently been purchased in the market and there are accordingly no available data as to its age or ancestry. It was apparently in perfect health at the time of its inoculation.

The tumor, as seen at autopsy, occupies the entire upper pole of the left kidney, forming a spherical mass 3 by 3.5 cm. in diameter, with a nodular and uneven surface, and completely covered by a fibrous capsule which is continuous with that of the kidney; the color of the growth is distinctly lighter than that of the kidney. Its cut surface is pale gray in hue, and shows numerous minute yellowish spots scattered throughout. The tumor is definitely outlined, being sharply separated from the tissues of the kidney not only by its color, but by the extension of the renal capsule, which entirely surrounds it. The mass is largely limited to the cortical area but by pressure it has greatly reduced the thickness of the medullary portion. A careful search fails to reveal evidences of metastasis.

Microscopically, the tumor corresponds closely to those described by Lubarsch (3) and by Bell and Henrici. The stroma, consisting of a rather loose fibrous tissue which extends irregularly throughout the mass, encloses groups of dark-staining, round or spindle shaped cells, in which may be distinguished clusters of epithelial elements, having a strong tendency to arrange themselves in the form of tubules. In a single mass of these dark staining cells, all stages in the formation of tubules may be seen, from those in which the elements are with difficulty distinguished as epithelial, to those in which the tubules are perfect and are lined by definite cuboidal epithelium. In those tubules which have become dilated and are more or less cystic, the epithelium has undergone a decided flattening. In many of the well-formed tubules, and in some that have become cystic, there is an accumulation of hyaline material closely resembling the so-called hyaline casts seen in the tubules in some types of nephritis.

In many places, small cell clusters may be seen, surrounded more or less completely by a single layer of flattened cells arranged in the form of a capsule; in some of these, there is a distinct capsular space between the cluster and its capsule, and these areas undoubtedly represent attempts at the formation of glomeruli. None are complete, however, and in none is there evidence of vascularization. Mitosis, which is mentioned by Nürnberger (4), was not seen in this case nor did it occur in the cases of Bell and Henrici.

The histological descriptions of the other cases recorded in the rabbit correspond closely with this, each recording the masses of darkly staining, undifferentiated cells including or surrounding the somewhat rudimentary tubules; and Bell and Henrici placed special emphasis upon the attempted formation of glomeruli. By comparison, these tumors are seen to resemble closely in their morphology the tissues of the normal embryonic kidney, and are thought by Bell and Henrici to arise entirely from such tissue.

Lubarsch drew attention to the fact that this neoplasm in the rabbit is similar to a new growth occurring in the kidneys of children, which has been described by Birch-Hirschfeld (5) and by Wilms (6); the former of these investigators believed that the masses of undifferentiated cells, the origin of which he ascribed to misplaced portions of the wolffian body, were sarcomatous in nature and that the tumor was properly to be called adenosarcoma. Wilms discovered that in many of his cases hyaline cartilage, as well as both striated and non-striated muscle fibers, were to be found, and he therefore described them as mixed tumors arising from misplaced remnants of the primitive myotome. All the renal tumors of the rabbit reported up to this time are strictly benign, being completely encapsulated and giving rise to no metastasis. In children, however, such growths may assume a high degree of malignancy.

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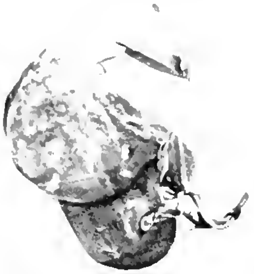
EXPLANATION OF PLATE

Fig. 1. The gross appearance of the tumor on sagittal section showing the capsule and the area from which the microscopical sections were made.

Fig. 2. Showing the masses of undifferentiated cells with more or less distinct tubule formation taking place.

Fig. 3. An area in which the tubules are more definitely found; some because of dilatation, are lined with flattened epithelium. Many of the tubules contain a material resembling hyaline casts.

Fig. 4. A rudimentary glomerulus showing a somewhat dilated capsular space with flattening of the cells of the outer layer of the capsule.



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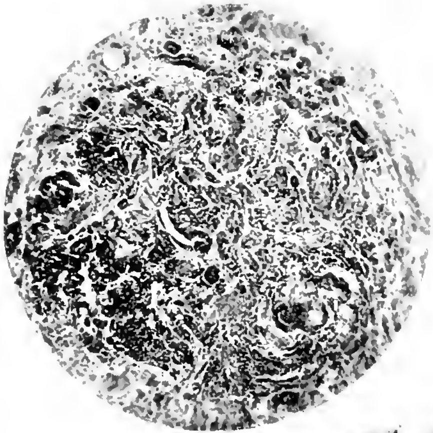

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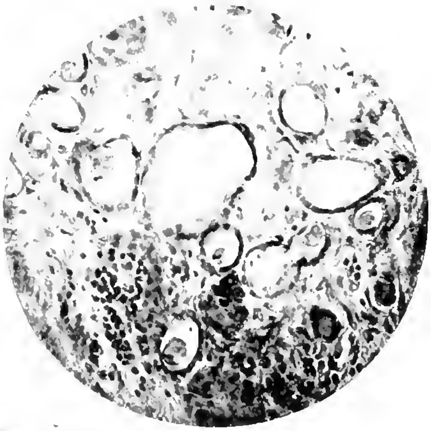

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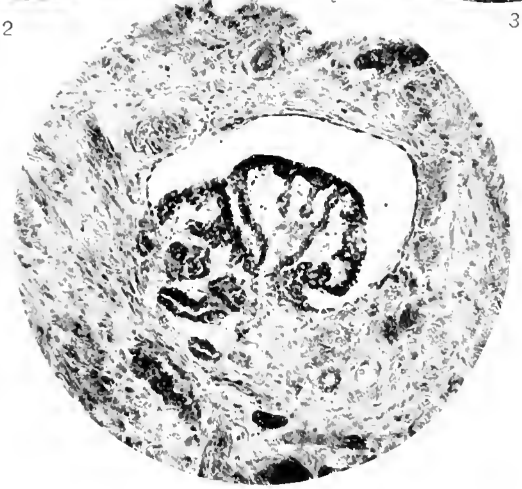

4

ON THE DISTRIBUTION OF THE IMMUNE STATE IN MICE[1]

SECOND COMMUNICATION ON HOMOLOGOUS IMMUNITY TO MALIGNANT MOUSE TUMORS

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The nature of cancer immunity or resistance (homologous) remains still obscure, although so many authors have endeavored to solve this problem. It must be due to the difficulty that we are not able to demonstrate any antibody in an immunized animal, especially in its serum, as is the case in bacterial immunity.

Ehrlich (1906) (1) has formulated the so-called "atreptic theory" in place of the "side chain theory" to explain this subject. However, Bashford (1913) (2) and others believe that the induced resistance must be caused by antibodies, although they have not been able to demonstrate their presence.

A first question to answer is whether the state of active resistance against cancer is localized or generalized; that is to say, whether immunity in mice produced by subcutaneous inoculation of mouse tumors or certain normal mouse tissues—mouse embryo-skin—is restricted to the subcutaneous tissues, or is extended over the whole body. Kraus, Ranzi and H. Ehrlich (1910) (3) investigated the subject by using rat tumor, and found that the tumor did not grow either in internal organs or in the peritoneal cavity of the immunized animals. Afterwards Woglom (1911) (4) asserted that the state of resistance in mice produced by subcutaneous inoculation of mouse embryo-skin is to be found in the kidney as well.

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[1] The author has not read the proof of this article.

In the present piece of work my plan was to test what influence exists on tumor cells deposited in the lungs of mice previously immunized by subcutaneous inoculation with mouse embryo-skin.

METHODS

The tumors employed were a mouse carcinoma and a polymorphous-cell mouse sarcoma. Their virulence is so great that the take is practically 100 per cent and their proliferative activity is so rapid that they produce from 2 to 3 grams three weeks after a subcutaneous inoculation of 0.03 cc.

The immunizing material used was the skin of mouse embryos; it was proved in a previous work that by a preliminary inoculation of this material satisfactory immunity against both tumors was obtained. Actually, 0.05 cc. of mouse embryo-skin was inoculated into a certain number of mice in the left axilla I have already published a paper entitled "On Sarcoma Immunity in Mice" (1916) (5), dealing with the intensity and duration of such an immunity. According to these experiments, a state of resistance sets in about the seventh day after inoculation of the immunizing material, and very soon afterwards attains to its maximum; at the end of the second week the immunity had diminished slightly. The fall is markedly greater at the end of the third week and continues from week to week. In the present case, two weeks after the subcutaneous inoculation of mouse embryo-skin the resistance of the animals was tested by an intravenous injection of the mouse tumor suspension.

For inoculation, 0.3 cc. of healthy tumor tissue was removed within ten to twenty-one days after inoculation, cut up under sterile conditions, and suspended in about 3 cc. of normal saline. This fine suspension was then drawn up into a 1 cc. all-glass syringe armed with a fine short glaucoma needle, and inoculated intravenously into a certain number of mice which were neither too young nor too old, after the method of Takahashi (1915) (6). The injection has to be made slowly, and it was found that 0.15 cc. was generally tolerated. In a few cases, however, death from embolism occurred. Three weeks after the injection the mice were killed, the lungs removed and preserved. They were then cut in serial sections and examined, so that even microscopic growths could not be missed.

For the purpose of comparison it is necessary to show in the following figure (fig. 1) the extent to which mice can be immunized against the subcutaneous inoculations of these two growths.

The charts show quite clearly that two weeks after treatment with 0.05 cc. of mouse embryo-skin, resistance against carcinoma stood at 93.3 per cent and against sarcoma at 80.0 per cent. The occurrence of tumors in the lungs of normal and immune mice after intravenous inoculation of the same tumors is shown in tables 1 and 2.

Carcinoma is thus seen to develop in 83.3 per cent of the control mice, while only one of the eight immunized mice (12.5 per cent) had growths in the lungs. In the case of sarcoma, the take of the tumor in immunized mice was only 20 per cent, while in the control mice it was 71.4 per cent, the difference, though not so great as with carcinoma, being still large.

The histological picture of the tumors which developed in the lungs of immunized mice presented no peculiarities.

From these experiments one must admit that the resistance produced by subcutaneous inoculation with mouse embryo-

TABLE 1

Tumors in lungs of mice three weeks after inoculation of 0.15 cc. saline suspension of mouse carcinoma

CONTROL		IMMUNE	
1	+	1	−
2	+	2	−
3	−	3	−
4	+	4	−
5	+	5	−
6	+	6	−
		7	−
		8	+
83.3 per cent (+)	16.7 per cent (−)	12.5 per cent (+)	87.5 per cent (−)

TABLE 2

Tumors in lungs of mice three weeks after inoculation of 0.15 cc. saline suspension of mouse sarcoma (polymorphous-cell)

CONTROL		IMMUNE	
1	−	1	−
2	+	2	−
3	+	3	−
4	+	4	+
5	+	5	−
6	+	6	+
7	−	7	−
		8	−
		9	−
		10	−
71.4 per cent (+)	28.6 per cent (−)	20.0 per cent (+)	80.0 per cent (−)

Three controls died too early to be included in the experiment.

skin is not only localized in the subcutaneous tissues but distributed also throughout the lungs of the animals.

Immunity to carcinoma (0.03 cc. of an emulsion) transplanted into the peritoneal cavity in mice which had been previously treated with mouse embryo-skin, was observed when the mice were examined three weeks after transplantation.

TABLE 3

The results of intraperitoneal inoculation of mouse carcinoma into immunized mice

CONTROL		IMMUNE	
1	+	1	+
2	+	2	+
3	+	3	+
4	+	4	+
5	+	5	−
6	+	6	−
7	+	7	−
8	−	8	−
9	+		
10	+		
90 per cent (+)	10 per cent (−)	50 per cent (+)	50 per cent (−)

Two immunes died too early to be included in the experiment.

As the table shows, the tumor failed to grow in the peritoneal cavity in 50 per cent of the mice immunized by subcutaneous inoculation of mouse embryo-skin, although the take of the tumor reached 90 per cent in control mice The resistance induced by subcutaneous inoculation is therefore shown to extend to the peritoneal cavity as well.

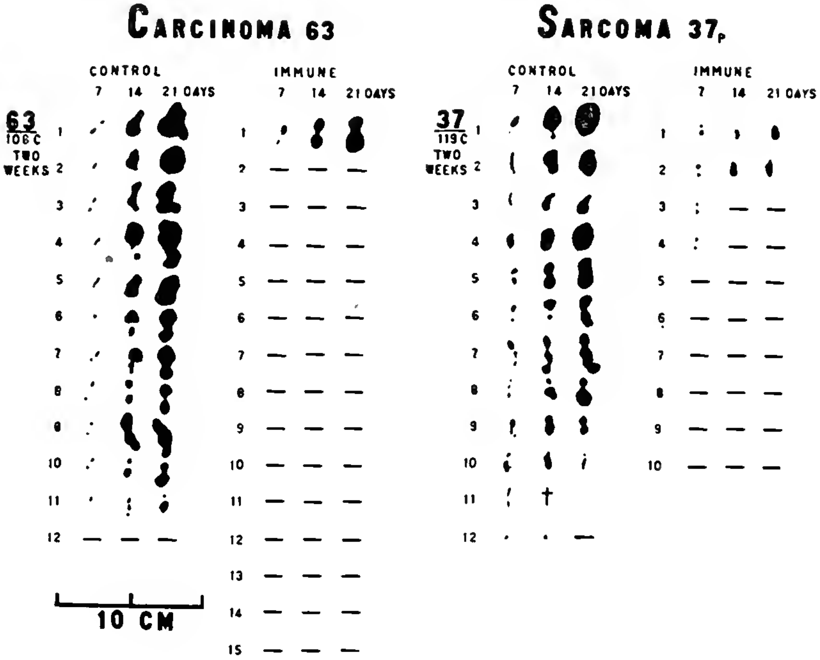

FIG. 1. First and third columns, normal untreated control mice. Second and fourth columns, immunized mice.

From these two series of experiments it would appear as if the lungs of animals immunized with mouse embryo-skin acquire a greater degree of immunity than the peritoneal cavity; for confirmation of this further investigation is necessary.

CONCLUSIONS

The immune state produced in mice by a single subcutaneous inoculation of mouse embryo-skin (0.05 cc.) has been shown to extend to an internal organ—the lungs, and to the peritoneal cavity. In other words, resistance is not localized, but is distributed all over the body of immunized animals. This fact allows the assumption of the presence in immunized animals of some substance which is able to cause resistance and which is distributed throughout the body of such animals.

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SULPHUR METABOLISM IN CARCINOMA

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Cario, in 1888, made a determination of the sulphuric acid output in cases of esophageal carcinoma, and found that the excretion is parallel to that of the nitrogen. On two occasions, he obtained figures which are disproportionately high, but he was unable to give an explanation of this phenomenon.

In 1892, Töpfer found that the urine of patients suffering from cancer contains a very large amount of "extractive substance," which was calculated by first determining the quantity of total nitrogen and then subtracting from this amount the sum of the nitrogen values for urea, uric acid, and ammonia of the same urine. Bondzynski and Gottlieb, five years later, reported that the nitrogen in oxyproteic acid in the urine is 2 to 3 per cent of the total urinary nitrogen. Salkowski, and Hess and Saxl, using different procedures, concluded that the oxyproteic acid of the alcohol precipitable substances is increased in the urine of human beings suffering from carcinoma.

Salkowski and Kojo, in a preliminary communication, have recently suggested several methods for the determination of colloidal nitrogen in the urine, and a year later Kojo published the results of a comparative study of the various procedures suggested in this connection. Kahn and Rosenbloom have studied the zinc-sulphate precipitable, colloidal nitrogenous material from the urine of normal subjects, as well as of carcinomatous patients, and have concluded that the amount of colloidal nitrogen is invariably increased in carcinoma; but they found that diseases like myocarditis, diabetes, leukemia, and anemia likewise give a high colloidal nitrogen index, and concluded, therefore, that this quantitative test is not specific for cancer. These authors have investigated, also, the amount of colloidal nitrogenous substances in the urine of a dog with a malignant neoplasm, using dialysis as a part of the method of analysis, and found that the quantity of colloidal nitrogen was much greater in the urine of the diseased dog than the amount present in the urine of normal dogs.

Volpe asserted that the colloidal nitrogen index is of especial value in cancer diagnosis, while Mancini, using the Salkowski method, found that there is an increased elimination of colloidal nitrogen in the urine of patients afflicted with cancer, but that this increase occurs also in pneumonia and pleurisy. Semionov reported that the colloidal nitrogen output is low in normal individuals and is increased in cancer patients, and concluded that although a normal index excludes the possibility of a malignant growth, the presence of an increased index is not specific for cancer. Konikov found that the average amount of colloidal nitrogen in the urine, as determined by the Salkowski-Kojo method is 1.68 per cent of the total nitrogen in normal cases, and 2.47 per cent in carcinomatous individuals. Of 73 cases of cancer investigated by him, only 9 showed a coefficient higher than 2.5 per cent.

According to Marcel, Labbé and Dauphin, and others, on the other hand, an increase in the urinary colloidal nitrogen is an index of a derangement of nitrogenous metabolism; and while it may serve to detect functional insufficiency in the liver, it is not at all specific for cancer. Carforio, also, concluded that a high colloidal nitrogen index is not pathognomonic of cancer, and Marriott and Wolf found that the colloidal nitrogen fraction of the urine is an inconstant value.

Salomon and Saxl have described a neutral sulphur reaction in the urine. Like all the other tests in this connection, it has given excellent results in some hands, but has proved valueless in others. The abnormal constituent in the urine of carcinomatous patients is a neutral sulphur fraction, the sulphur of which can be split off by means of hydrogen peroxide, oxidized, and determined as barium sulphate. Positive urines yield 0.010 to 0.018 gram of barium sulphate from this fraction, for

100 cc. of urine. Of 41 carcinoma cases examined by Salomon and Saxl, 30 were positive, 4 faintly positive, 1 questionable, and 6 negative. Of 182 normal urines, 6 were positive, 3 faintly positive, 1 questionable, and 172 negative.

Petersen divided his cases into three classes (a) Clinically non-cancerous suspects: of 26 patients examined, 25 gave a negative Salomon and Saxl neutral sulphur reaction. (b) Clinically cancer suspects: of 20 cases examined, 5 were negative, 2 alternately positive and negative, and 13 positive. (c) Manifest cancer: of 19 cases, 17 always gave a good positive reaction; the two negative were icteric and cachectic. Dozzi found that the test was invariably negative in all his patients free from cancer or tuberculosis, but that the frequency of the positive response in tuberculous patients detracted from its value as a sign of cancer, although cancer is rarely mistaken for tuberculosis. The only cancer cases that gave negative results were those in which the cancer had been excised. Murachi, also, found an increase in the neutral sulphur in the urine of cancer patients. The coefficient, according to him, may be 3.8 per cent of the total sulphur.

In contrast to the foregoing, Pribram found that only 60 per cent of cancer patients gave a positive Salomon-Saxl test and that the test is, therefore, far from specific. Alekseev came to a similar conclusion. Mazzitelli has studied this test in 50 cases of cancer, with and without cachexia. Of 18 cases of the latter variety, the test was positive in 14; but it was positive also in 8 of 10 cases of tuberculous cachexia, and in 16 out of 23 cases of cachexia of various origins, including 11 with cancer and 4 with tuberculosis. Greenwald concluded that this test is of no value in the diagnosis of cancer, and Kaldeck regarded it as of limited significance.

In a study of the sulphur in the urine, Harnack and Kleine found that in cancer about 23.4 per cent to 24.5 per cent is in the form of neutral sulphur, a distinct increase above the normal, and Reale and Velardi obtained similar results. In the experiments of F. Müller, the neutral sulphur fraction in the urine was 22.1 per cent of the total sulphur (average of three estimations), while Weiss reported that the unoxidized sulphur excretion in carcinoma amounted to from 20.3 to 36.1 per cent of the total sulphur.

Rosenbloom and Stadtmüller drew the following conclusions from their study of the sulphur excretion in malignancy:

1. The lowest total (average) excretion of sulphur—0.88 gram per day—was found in a series of 13 cases of carcinoma.
2. The same series showed also the lowest average neutral sulphur excretion—0.20 gram per day.
3. The proportion of neutral sulphur to the total sulphur in this group is considerably higher than the normal proportion of total sulphur excreted as neutral sulphur.
4. In the light of their own experience, they regard it as a precarious undertaking to diagnose a malignant tumor on the basis of the absolute or relative amount of the neutral sulphur excreted in the urine, or from the daily excretion of the total sulphur.

It may be noted that in the 13 cases of cancer which they investigated, the neutral sulphur of the urine was consistently high both in amount and in proportion to the total sulphur. In these cases, the lowest neutral sulphur excretion was 0.05 gram, and the highest 0.76 gram. In relation to the proportion of total sulphur excreted as neutral sulphur, the lowest was 6.2 per cent and the highest 67.9 per cent. While the average total sulphur excreted was 0.88 gram, the average neutral sulphur was 0.020 gram or 23.1 per cent of the total sulphur.

The author, in collaboration with Goodridge, has made a comparison of the incidence of both the colloidal nitrogen test and the Salomon-Saxl test in carcinoma. The following tables summarize the results which we obtained in normal and cancerous patients and in patients afflicted with non-cancerous diseases.

The first table of Goodridge and the author shows the results obtained in normal subjects. The nitrogen value for the zinc-sulphate precipitate, as compared with those for total nitrogen, varied from 1.25 per cent as a minimum, to 2.15 per cent as a maximum, with an average of 1.67 per cent. This agrees with the results obtained by Salkowski and Kojo, and by Einhorn, Kahn, and Rosenbloom, who obtained respectively averages of 1.75 per cent and 1.9 per cent. Of 22 urines examined, 10 gave a precipitate by the Salomon and Saxl method that was so light as not to be weighable. The other 12 cases gave sulphate precipitates which varied between 1.22 per cent of the total sulphur as a minimum, and 2.14 per cent of total sulphur as a maximum. In general, the Salomon and Saxl test was negative in all cases in which the neutral sulphur was less than 2 per cent of the total sulphur. Considering it from this point of view, 90.0 per cent of normal cases gave a negative Salomon and Saxl reaction.

TABLE 1

Data pertaining to normal cases

NUMBER	NAME	DIAGNOSIS	TOTAL N IN 100 CC. URINE, IN GRAMS	COLLOID-N IN 100 CC URINE, IN GRAMS	PER CENT COLLOID-N OF TOTAL N	TOTAL S IN 100 CC. URINE, IN GRAMS	SALOMON-SAXL NEUTRAL-S IN 100 CC. URINE IN GRAMS	PER CENT NEUTRAL-S IN TOTAL S
1	A. I.	Normal	0.7459	0.01006	1.35	0.112	0.0019	1.72
2	A. I.	Normal	0.7875	0.0098	1.25	0.109	0.0018	1.65
3	J. S.	Normal	0.8132	0.0109	1.84	0.097	Not weighed	Less than 1
4	M. K.	Normal	0.7986	0.0167	2.10	0.124	0.0027	2.07
5	D. F.	Normal	0.9178	0.0164	1.74	0.171	0.0033	1.94
6	J. S.	Normal	0.9356	0.0175	1.87	0.195	0.0026	1.34
7	S. H.	Normal	0.9471	0.0136	1.44	0.172	Not weighed	Less than 1
8	R. L.	Normal	0.7344	0.0118	1.62	0.155	Not weighed	Less than 1
9	B. C.	Normal	0.5467	0.0103	1.90	0.137	0.0017	1.22
10	J. H.	Normal	0.8264	0.0158	1.92	0.208	0.0044	2.14
11	D. F.	Normal	0.8326	0.0146	1.75	0.170	Not weighed	Less than 1
12	W. S.	Normal	0.8521	0.0113	1.33	0.115	Not weighed	Less than 1
13	M. K.	Normal	0.7287	0.0153	2.10	0.110	0.0025	1.75
14	M. K.	Normal	0.9812	0.0210	2.15	0.135	0.0023	1.90
15	J. G.	Normal	0.9245	0.0138	1.50	0.152	0.0024	1.62
16	A. P.	Normal	0.8352	0.0129	1.55	0.162	Not weighed	Less than 1
17	V. L.	Normal	0.6992	0.0118	1.70	0.178	Not weighed	Less than 1
18	B. H.	Normal	0.9272	0.0124	1.34	0.096	Not weighed	Less than 1
19	D. R.	Normal	0.9817	0.0124	1.35	0.087	Not weighed	Less than 1
20	S. H.	Normal	0.8228	0.0116	1.42	0.135	0.0025	1.90
21	R. L.	Normal	0.8298	0.0142	1.72	0.109	0.0020	1.85
22	M. B.	Normal	0.8218	0.0156	1.90	0.175	Not weighed	Less than 1

In the results for 59 urinary examinations of cases of cancer, the colloidal-nitrogen per cent was generally increased to as

TABLE 2

Data pertaining to cancer cases

NUMBER	NAME	DIAGNOSIS	TOTAL N IN 100 CC. URINE, IN GRAMS	COLLOID-N IN 100 CC. URINE, IN GRAMS	PER CENT COLLOID-N OF TOTAL N	TOTAL S IN 100 CC. URINE, IN GRAMS	SALOMON-SAXL S NEUTRAL IN 100 CC. URINE, IN GRAMS	PER CENT NEUTRAL S IN TOTAL S
23	T. A.	Cancer of uterus	0.9756	0.0419	4.3	0.085	0.0031	3.7
24	M. W.	Gastric cancer	1.1070	0.0636	5.75	0.087	0.0035	4.1
25	F. C.	Gastric cancer	1.1950	0.0652	4.62	0.108	0.0041	3.8
26	A. R.	Cancer of breast	1.2104	0.0568	4.7	0.152	0.0045	2.9
27	S. G.	Gastric cancer	0.9260	0.0361	3.9	0.095	Not weighed	Less than 1
28	T. S.	Cancer of liver	0.5762	0.0247	4.3	0.104	0.0035	3.4
29	T. A.	Cancer of uterus	1.3550	0.0469	4.2	0.087	0.0032	3.7
30	M. W.	Gastric cancer	0.8722	0.0305	3.5	0.1055	0.0025	2.5
31	S. G.	Gastric cancer	0.9128	0.0447	4.9	0.1243	0.0045	4.4
32	A. R.	Cancer of breast	1.0424	0.0458	4.4	0.1175	0.0037	3.2
33	A. G.	Cancer of rectum	0.4728	0.0212	4.5	0.1480	0.0050	3.4
34	C. J.	Cancer of cervix	1.1307	0.0431	4.7	0.0875	0.0030	3.5
35	W. J.	Gastric cancer	0.9246	0.0388	4.2	0.0986	0.0036	3.7
36	B. M.	Cancer of liver	1.1108	0.0377	3.4	0.097	0.0031	3.2
37	K. B.	Cancer of liver	0.8229	0.0427	5.2	0.1452	0.0062	4.3
38	E. F.	Cancer of stomach	1.1055	0.0608	5.5	0.1445	0.0059	4.1
39	B. J.	Cancer of pancreas	1.1782	0.0494	4.2	0.1378	0.0060	4.4
40	E. M.	Cancer of stomach	1.1363	0.0441	3.8	0.1025	0.0043	4.2
41	B. M.	Cancer of liver	0.8912	0.0427	4.8	0.1644	0.0070	4.0
42	C. J.	Cancer of cervix	0.7755	0.9334	4.3	0.1552	0.0067	4.5
43	P. B.	Cancer of uterus	0.5737	0.0252	4.4	0.1275	0.0049	4.1
44	M. G.	Cancer of stomach	0.9345	0.0345	3.7	0.09865	0.0035	3.4
45	E. F.	Cancer of stomach	0.8548	0.0435	5.1	0.1143	0.0038	3.5
46	M. W.	Gastric cancer	0.9845	0.0502	5.1	0.0975	0.0026	2.7
47	F. O.	Cancer of pelvis	0.9642	0.0424	4.4	0.0956	0.0039	4.2
48	M. F.	Cancer of cervix	0.8750	0.0411	4.7	0.1140	0.0031	2.9
49	C. J.	Cancer of cervix	0.6437	0.0270	4.2	0.1231	0.0037	3.1
50	R. W.	Cancer of rectum	0.8821	0.0379	4.3	0.1242	0.0041	3.4
51	C. S.	Cancer of appendix	1.0632	0.0404	3.8	0.0983	0.0031	3.2
52	R. W.	Cancer of rectum	1.0452	0.0377	3.7	0.0875	0.0031	3.5
53	C. S.	Cancer of appendix	1.2371	0.0507	4.1	0.0844	0.0031	3.7
54	Z. H.	Cancer of stomach	1.1685	0.0561	4.8	0.0847	0.0026	3.1
55	A. T.	Cancer of stomach	0.7325	0.0370	5.05	0.0934	0.0031	3.4
56	I. S.	Cancer of liver	0.4510	0.0237	5.2	0.0895	0.0029	3.3
57	A. I.	Cancer of liver	1.4784	0.0784	5.3	0.0880	0.0034	4.2
58	R. A.	Cancer of breast	0.6905	0.0359	5.2	0.0975	0.0041	4.3
59	G. T.	Cancer of esophagus	0.9075	0.0472	5.2	0.1302	0.0043	4.1
60	G. F.	Cancer of esophagus	0.6470	0.0349	5.4	0.0888	0.0031	3.6

TABLE 2—*Continued*

NUMBER	NAME	DIAGNOSIS	TOTAL N IN 100 CC. URINE, IN GRAMS	COLLOID—N IN 100 CC. URINE, IN GRAMS	PER CENT COLLOID—N OF TOTAL N	TOTAL S IN 100 CC. URINE, IN GRAMS	SALOMON-SAXL NEUTRAL S IN 100 CC. URINE, IN GRAMS	PER CENT NEUTRAL S IN TOTAL S
61	M. B.	Cancer of intestine	0.6984	0.0356	5.1	0.1207	0.0055	4.6
62	B. J.	Cancer of pancreas	0.9750	0.0536	5.5	0.1405	0.0053	3.8
63	E. O.	Cancer of esophagus	1.1750	0.0564	4.8	0.0995	0.0030	3.2
64	D. G	Cancer of breast	0.8705	0.0409	4.7	0.0894	0.0050	4.5
65	F. G.	Cancer of breast	0.6095	0.0298	4.75	0.1114	0.0051	4.7
66	G. H.	Cancer of uterus	0.5276	0.0243	4.6	0.1237	0.0039	3.3
67	I. S.	Cancer of liver	0.7654	0.0260	3.4	0.1047	0.0040	3.9
68	Z. H.	Cancer of stomach	0.8505	0.0366	4.3	0.0997	0.0038	3.8
69	A. T.	Cancer of stomach	0.9472	0.0360	3.8	0.0896	0.0029	3.3
70	B. J.	Cancer of pancreas	1.1560	0.0541	4.7	0.0945	0.0035	4.8
71	D. S.	Cancer of stomach	1.423	0.0448	3.2	0.1144	0.0047	4.2
72	T. A.	Cancer of uterus	1.2025	0.0408	3.4	0.0795	0.0024	3.1
73	M. W.	Gastric cancer	0.8701	0.0296	3.4	0.0994	0.0034	3.5
74	F. C.	Gastric cancer	0.7575	0.0271	3.6	0.0774	0.0028	3.6
75	A. G.	Cancer of rectum	1.3645	0.0525	4.6	0.0985	0.0033	3.4
76	C. J.	Cancer of cervix	0.6143	0.0248	3.4	0.0863	0.0031	3.6
77	W. J.	Gastric cancer	1.2380	0.0516	4.2	0.0784	0.0035	4.5
78	B. M.	Cancer of liver	0.9522	0.0432	5.6	0.0952	0.0027	2.8
79	E. F.	Cancer of stomach	1.202	0.0528	4.4	0.1205	0.0040	3.7
80	M. G.	Cancer of stomach	0.7540	0.0337	4.5	0.1104	0.0041	3.8
81	K. B.	Cancer of larynx	0.6884	0.0353	5.2	0.0948	0.0039	4.4

high as 5.75 per cent of the total nitrogen, the minimum being 3.4 per cent. Fifty-eight of the 59 cases of cancer gave a positive Salomon and Saxl reaction.

Forty-seven cases of diseases other than cancer were studied. We obtained positive results with the colloidal-nitrogen estimations in cases of myocarditis, diabetes, and syphilis. The colloidal nitrogen was constantly increased in amount in diabetics. Wallace, basing his conclusion on the findings in only two cases, stated that this increase is not constant and that there is no relationship between the colloidal-nitrogen output and the severity of the diabetes. Tuberculosis and other diseases gave negative results. On the other hand, tuberculosis, hemophilia, pernicious anemia, and atrophic cirrhosis of the liver, gave positive Salomon and Saxl neutral-sulphur reactions, whereas the other diseases reacted negatively.

TABLE 3

Data pertaining to non-cancerous cases

NUMBER	NAME	DIAGNOSIS	TOTAL N IN 100 CC. URINE, IN GRAMS	COLLOID-N IN 100CC URINE, IN GRAMS	PER CENT COLLOID-N OF TOTAL N	TOTAL S IN 100 CC. URINE, IN GRAMS	SALOMON-SAXL NEUTRAL-S IN 100 CC. URINE, IN GRAMS	PER CENT NEUTRAL S IN TOTAL S
82	L. S.	Pulmonary tuberculosis	0.872	0.0117	1.35	0.1409	0.0047	3.4
83	A. H.	Nephritis	0.695	0.0118	1.75		Not weighed	Less than 1
84	L. L.	Nephritis	0.723	0.0144	2.0		Not weighed	Less than 1
85	P. B.	Nephritis	0.646	0.0115	1.8		Not weighed	Less than 1
86	C. H.	Myocarditis	1.078	0.0363	3.4		Not weighed	Less than 1
87	G. J.	Myocarditis	1.055	0.0221	2.1		Not weighed	Less than 1
88	S. B.	Typhoid	1.072	0.0139	1.3		Not weighed	Less than 1
89	S. E.	Typhoid	0.9465	0.0118	1.25		Not weighed	Less than 1
90	B. I.	Typhoid	0.6435	0.0081	1.35	0.1875	0.0031	1.7
91	H. R.	Empyema	0.7281	0.0122	1.7		Not weighed	Less than 1
92	B. R.	Empyema	0.8253	0.0139	1.75		Not weighed	Less than 1
93	J. B.	Empyema	0.7612	0.0083	1.1		Not weighed	Less than 1
94	A. I.	Endarteritis, obliter.	0.6895	0.0108	1.6		Not weighed	Less than 1
95	H. W.	Endarteritis, obliter.	0.7321	0.0102	1.4		Not weighed	Less than 1
96	A. K.	Endarteritis, obliter.	0.8218	0.0147	1.8		Not weighed	Less than 1
97	M. R.	Endarteritis, obliter.	1.0878	0.0097	0.9		Not weighed	Less than 1
98	J. R.	Sarcoma of leg	1.046	0.0468	4.5		Not weighed	Less than 1
99	C. S.	Leukemia	1.0975	0.0239	2.2		Not weighed	Less than 1
100	child	Hemophilia	0.784	0.0109	1.4	0.1482	0.0033	2.4
101	C. F.	Pernicious anemia	1.095	0.0130	1.2	0.1843	0.0045	2.5
102	R. K.	Atrophic cirrhosis	0.5965	0.0106	1.8	0.1077	0.0029	2.8
103	F. G.	Atrophic cirrhosis	0.7234	0.0101	1.4	0.1255	0.0025	2.1
104	A. E.	Pneumonia	0.6546	0.0111	1.7		Not weighed	Less than 1
105	R. E.	Pneumonia	1.0725	0.0161	1.5		Not weighed	Less than 1
106	B. B.	Pneumonia	1.1347	0.0125	1.1		Not weighed	Less than 1
107	H. S.	Pneumonia	1.1485	0.0161	1.4		Not weighed	Less than 1
108	J. M.	Diabetes	1.0953	0.0466	4.25		Not weighed	Less than 1
109	J. A.	Diabetes	1.2075	0.0465	3.75		Not weighed	Less than 1
110	B. S.	Diabetes	0.9642	0.0501	5.2		Not weighed	Less than 1
111	I. B.	Diabetes	1.2007	0.0552	4.6		Not weighed	Less than 1
112	I. B.	Diabetes	0.6435	0.0290	4.5		Not weighed	Less than 1

TABLE 3—*Continued*

NUMBER	NAME	DIAGNOSIS	TOTAL N IN 100 CC. URINE, IN GRAMS	COLLOID-N IN 100 CC. URINE, IN GRAMS	PER CENT COLLOID-N OF TOTAL N	TOTAL S IN 100 CC. URINE, IN GRAMS	SALOMON-SAXL NEUTRAL-S IN 100 CC. URINE, IN GRAMS	PER CENT NEUTRAL S IN TOTAL S
113	A. K.	Syphilis	0.7114	0.0263	3.7		Not weighed	Less than 1
114	H. H.	Syphilis	0.7227	0.0296	4.1		Not weighed	Less than 1
115	S. H.	Syphilis	0.5835	0.0222	3.8		Not weighed	Less than 1
116	M. F.	Gastric ulcer	0.6444	0.0077	1.2		Not weighed	Less than 1
117	M. M.	Gastric ulcer	0.9007	0.0126	1.4	0.1586	0.0036	2.4
118	B. W.	Gastric ulcer	0.8767	0.0075	0.85	0.1755	0.0042	2.5
119	C. Z.	Gastric ulcer	0.6114	0.0109	1.7		Not weighed	Less than 1
120	J. J.	Gastric ulcer	0.6275	0.0100	1.6		Not weighed	Less than 1
121	R. H.	Gastric ulcer	0.8649	0.0130	1.5		Not weighed	Less than 1
122	child	Chorea	0.9653	0.0116	1.2		Not weighed	Less than 1
123	child	Chorea	0.7556	0.0128	1.7		Not weighed	Less than 1
124	P. B.	Endocarditis	1.0755	0.0258	2.4		Not weighed	Less than 1
125	E. L.	Tuberculosis of glands	0.6443	0.0141	2.2	0.1641	0.0068	4.3
126	N. R.	Pulmonary tuberculosis	0.7627	0.0137	1.8	0.1974	0.0072	3.8
127	H. F.	Pulmonary tuberculosis	1.2005	0.0281	1.4	0.1722	0.0063	3.7
128	N. K.	Pulmonary tuberculosis	0.9234	0.0139	1.5	0.1645	0.0046	2.9

Goodridge and the author concluded that positive results with either the colloidal-nitrogen test or the neutral-sulphur test, alone, are not indicative of carcinoma. When performed conjointly on urine of the same case, however, positive results with both methods are strongly indicative of malignancy.

The technic employed for these two tests was as follows:

Colloidal-nitrogen. The urine was first tested for coagulable protein, which if found, was removed by means of heat coagulation, with addition to the boiling liquid of a few drops of dilute acetic acid. To 100 cc. of a mixed, filtered, twenty-four hour specimen of urine, zinc sulphate was added in sufficient quantity to effect saturation. The saturated liquid was allowed to stand for twenty-four hours, then filtered through ashless paper, and the precipitate washed several times on the paper with saturated zinc sulphate solution, to remove nitrogenous substances adherent to the precipitate. The paper and precipitate were then placed in a Kjeldahl flask and the nitrogen content determined by the Kjeldahl method. The total nitrogen in 5 cc. of urine was also determined by the Kjeldahl method. The ratio of the nitrogen in the zinc sulphate precipitate to the total urinary nitrogen was computed.

Salomon-Saxl neutral sulphur test. One hundred and fifty cubic centimeters of urine, freed from coagulable protein by heat and acid, were diluted with 100 cc. of water. A mixture of 100 cc. of a saturated aqueous solution of barium hydroxid and 50 cc. of a saturated aqueous solution of barium chlorid was added, the liquid filtered, and the filtrate tested with barium to see if precipitation had been complete. In order to remove the ethereal sulphates, 300 cc. of the filtrate were treated with 30 cc. of concentrated hydrochloric acid solution, and boiled for fifteen minutes in an Erlenmeyer flask, using a funnel condenser. The flask was then placed on a water-bath for twenty-four hours. Of the clear filtrate, 200 cc. were mixed with 3 cc. of hydrogen peroxide (perhydrol, Merck), and boiled for fifteen minutes with a funnel condenser. After boiling, the liquid was transferred to a conical graduate, where at the end of 6 hours, the amount of precipitate was observed. Antipyrin and creosote medication interferes, according to certain authors, with this test.

Saxl studied the Salomon-Saxl test in cases of cancer and concluded that:

1. The disturbances of protein metabolism in cancerous individuals have a similarity with those observed after feeding sulphocyanates to normal human beings; in both conditions there is an increased excretion of ammonia, neutral sulphur, and oxyproteic acids in the urine; there is also an increase (in both conditions) of the easily oxidizable substance in the urine.
2. Quantitative estimation of sulphocyanate in the urine shows it increased in cancer cases; the values here are not to be observed in any other disease.
3. The excretion of sulphocyanate has no relation to the nutrition, anemia, or cachexia; fever increases the sulphocyanate output, but not to the same extent that cancer does.

4. The Salomon-Saxl neutral sulphur test of the urine of cancer patients is due to an increase in the sulphocyanate output in the urine of such patients.

The following table shows the results which Saxl obtained:

TABLE 4

DISEASE	VOLUME URINE IN CUBIC CENTIMETERS	KSCN IN TOTAL URINE, IN GRAMS	KSCN IN 100 CC. URINE IN GRAMS	SALOMON-SAXL TEST
Cancer, stomach	1,300	0.1964	0.0157	Positive
Cancer, peritoneum	1,200	0.198	0.0165	Positive
Cancer, stomach	650	0.1458	0.0224	Positive
Cancer, stomach	350	0.0746	0.0213	Positive
Cancer, liver	600	0.1564	0.0260	Positive
Cancer, stomach	1,000	0.1124	0.0112	Negative
Cancer, esophagus	500	0.1024	0.0205	Positive
Cancer, stomach	1,050	0.2045	0.0194	Positive
Cancer, stomach	1,200	0.1467	0.0122	Negative
Cancer, stomach	1,400	0.1084	0.0077	Negative
Myelogenous leukemia	1,400	0.0987	0.0071	Negative
Pneumonia	700	0.0805	0.0115	Negative
Heart disease	450	0.0584	0.0137	Negative
Cirrhosis liver	1,200	0.0506	0.0042	Negative
Tuberculous peritonitis	1,100	0.0558	0.0055	Negative
Ulcer, stomach	1,400	0.0564	0.0041	Negative
Ulcer, stomach	800	0.0325	0.0041	Negative
Lung, tuberculosis	1,000	0.1365	0.0136	Doubtful
Sepsis (fever)	1,100	0.1122	0.0120	Negative
Bronze cirrhosis	3,000	0.2024	0.0067	Negative
Chronic nephritis	1,000	0.0822	0.0082	Negative
Cirrhosis, liver	700	0.0840	0.0120	Negative
Angina (fever)	1,000	0.0882	0.0088	Negative

Marendusso has recently denied that the Salomon-Saxl test has any significance for cancer. He found that by adding a catalytic agent like manganese dioxide, he could increase the so-called neutral sulphur of the urine. Malan has obtained similar results, but Pasetti, on the other hand, has obtained more encouraging data.

The Weisz reaction which, according to its originator, is a test for the urochromogen in the urine, going hand in hand with the neutral sulphur fraction, and is supposedly of such frequent occurrence in tuberculosis, was tested for by the author in 63 cases of malignancy. A positive result was obtained in 4 cases.

SUMMARY

In carcinoma there is generally observed an increase in the percentage of neutral sulphur in the urine. This may be ascribed to a lessened power of oxidation or to an increased destruction of tissue with the failure of the organism to oxidize the broken down products to the sulphate (oxidized) state.

The colloidal nitrogenous substances are increased in the urine of cancer patients, as well as in the urine of anemic, diabetic, and syphilitic individuals, etc. It has been the experience of the author that the determination of this fraction in conjunction with the Salomon-Saxl test for the so-called "neutral sulphur" is of great aid in the diagnosis of carcinoma if both of these tests are positive. A negative result with either the Salkowski-Kojo test or the Salomon-Saxl test detracts very much from the significance of the results so far as carcinoma is concerned.

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TRAUMATIC RHABDOMYOSARCOMA FOLLOWING SUCCESSIVE FRACTURES OF THE FEMUR

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It is for two chief reasons that this tumor, which came under observation at the Memorial Hospital, is reported. Clinically it is of interest in that it had developed apparently in a femur which had twice been the seat of a fracture, and which later had been treated for periostitis. Pathologically it is noteworthy in that its morphology differs strikingly from that of a bone sarcoma, and that it must be regarded as a tumor arising in muscle tissue. That injuries bear some causal relation to the production of a certain percentage of sarcomas has been widely accepted. For instance, in a compilation of 800 cases, Löwenthal (1) noted a history of trauma in 316. Coley (2) obtained a definite history of an antecedent injury in 46 of his 170 cases of sarcoma. Interesting in this connection is it, that in two of Coley's cases the tumor had developed at the site of a fracture of the femur and clavicle respectively, the tumor in both cases being a bone sarcoma. While undoubtedly most tumors that develop at the site of a fracture are sarcomas arising from bone or periosteum, the possibility that such a tumor may have had its origin in the surrounding muscle should nevertheless be considered, because in a fracture the neighboring tissues are also injured.

The following case represents such a malignant new growth of the muscles, developing on a traumatic basis.

History. The patient, H. F., a married man, age 48, was admitted to the hospital, October 16, 1916. He complained of a swelling of the right thigh, associated with some pain and inability to use the right leg. The patient had had a long history of injuries to the right femur dating back to his fourth year, at which time he fell and broke the bone. The fracture united, and he had good use of the leg until the age of 11, when he broke this femur again, whether at the former site or not, is not known. The fracture, however, united; and from that time until the age of 21 he enjoyed perfect function in the limb, except that it seemed to tire more quickly. At the age of 21 the patient is said to have had a periostitis, which developed at the seat of the old fracture. He was not operated upon at that time, but was treated medically for awhile. From that time up to the present, he has always had to use crutches. His condition remained about the same till June, 1916, when he noticed an enlargement at the site of the old fracture. He began to have considerable pain in the leg, and was unable to use it at all. The tumor gradually enlarged, and on August 4, 1916, he was operated on for a sequestrum, and bone was curetted out. In addition he had eight X-ray treatments. The incision wound healed fairly well, but the tumor gradually reappeared.

Physical examination. At the time of his admission to the hospital he had a swelling situated 10 cm. below the trochanter of the right femur, and covering the anterior and outer aspects of the thigh. The mass was hard, though somewhat resilient on pressure, smooth, fusiform, and apparently firmly attached to the bone. The overlying skin was slightly red and dry.

An X-ray examination on October 17, showed an extensive, localized destruction of the right femur. The shadow was reported as being "not characteristic of periosteal sarcoma."

After ten days' preliminary treatment with Coley's fluid, and two applications of radium, the right leg was disarticulated at the hip-joint on October 31.

Gross appearance of tumor. Situated on the anterior and outer aspects of the ununited ends of a fracture of the femur, at the junction of the upper and middle thirds of the shaft, there is a moderately hard mass, varying from 2 to 4 cm. in thickness. It is situated within the muscle, next to the bone, the line of separation between tumor tissue and bone being very sharp. It has a longitudinal dimension of 7 to 10 cm. The ends of the fracture on the posterior aspect are overriding about 0.5 cm., and on the anterior aspect there is a loss of bone, measuring 3 by 5 cm. in area, so that the ends of the fracture are separated on this aspect by this distance. Through this gap between the ends of the fragments, the tumor extends directly into the medullary cavity. The only evidence of remaining callus formation is on the posterior aspect, where the lower end of the upper fragment shows a fusiform thickening, entirely distinct from the tumor. Everywhere else the ends of the fragments appear thin and smooth, as if the bone were undergoing absorption, as sometimes happens in a fracture which has failed to unite.

On section, the tumor is seen to be, on the whole, well demarcated by a thin capsule from the normal muscle; at some points, narrow bundles of fibers extend from the margin of the tumor into the growth, and are gradually lost. The tumor is friable and can easily be broken up into short bundles of fibers, corresponding to the fascicular arrangement distinguishable in the gross. The color on section is grey, with numerous patches of dark red or brown hemorrhagic areas, especially numerous just within the outer edge of the tumor.

Microscopical. Very striking are the interlacing bundles of cells, suggesting bundles of muscle fibers. These elements are exceedingly anaplastic, but in general are long and fusiform. They are distinctly acidophile, staining deeply with eosin. A good proportion of them are mononuclear or multinuclear giant-cells. These latter are also elongated as a rule, with tapering or serrated ends.

The nucleus is, in general, rod-shaped, with blunted ends and coarsely granular chromatin, and often contains a distinct nucleolus. Scattered throughout the tumor are leucocytes and red blood cells.

Cross-striations could not be demonstrated in any of the tumor cells. The large spindle-shaped, acidophile elements just described, have, however, with their interlacing fascicular arrangement, a strong resemblance to bundles of smooth muscle fibers, or to young undifferentiated voluntary muscle fibers. Moreover, in some places the cells resemble somewhat those seen in regeneration of striated muscle.

A connective tissue capsule separates the tumor from the surrounding muscle.

With reference to the proper classification of this tumor, it is necessary first to consider briefly the attempt that has been made to classify myosarcomas in general. In 1913, Kuettner (3) in "Die Chirurgie der quergestreiften Muskulatur" collected 130 cases of primary muscle sarcomas which had been reported in the literature up to that time, and included in this report 16 of his own. Since then several additional cases have been reported (4, 5, 6, 7). Depending upon the histological structure from which they take origin, these sarcomas have been divided into two main groups, one containing those which take their origin from the fascias and aponeuroses, and the other group those derived from the muscle fiber itself; the former are relatively the most frequent. With regard to the question as to what part the striated muscle itself can play in the formation of tumors, there seems to be some uncertainty. The vast majority of neoplasms containing striated muscle fibres are those occurring in the ovary, testis, kidney, and other parts of the body, i.e., the mixed tumors or teratomas. Here the striated muscle fiber occurs in combination with one or more other tissues, and is thought to be derived either from embryonic muscle fiber rests, or from undifferentiated tissue capable of producing striated muscle cells. Such tumors are not uncommon. On the other hand, neoplasms derived from adult voluntary muscle fibers, in which the tumor cells retain the striations—the rhabdomyomas—are very uncommon. Of the 16 cases reported up to 1913 as having had such an origin, Kuettner rejects 6 as being positively not rhabdomyomas, and regards the remaining 10 as being still disputable. Then, finally, there appears to be a group of tumors, evidently exceedingly uncommon, derived from adult striated muscle fibers, in which the tumors are composed not of adult striated fibers, but of non-striated, highly anaplastic cells, and of numerous muscle giant-cells. To this group the name "rhabdomyosarcoma" has been applied. To those collected by Kuettner should be added the tumor reported by Adami, (8) which occurred in a fish. It was composed wholly of multinucleate cells, derived from muscle tissue,—a "pure giant-celled rhabdomyosarcoma." Probably also the tumors reported by Scott-Carmichael (4) as being sarcomas of both brachial biceps muscles were rhabdomyosarcomas.

As was indicated in the preceding description of the tumor reported in this paper, its characteristic features are in favor of its being, not a sarcoma of the bone or periosteum, but a tumor arising from the striated muscles surrounding the old fracture. It would likewise, on that account, have to be called a rhabdomyosarcoma. It was a tumor, which, although situated next to the bone, was still easily separated from the shaft, and invaded the medullary cavity only between the ends of the ununited fracture. The bone itself was everywhere either quiescent, or, as around the ends of the fracture, undergoing absorption. The growth itself was made up of interlacing bundles, as verified by the microscope. The cells themselves were different from cells of a bone sarcoma, either periosteal or medullary, in that they were long, acidophile cells with a type of nucleus generally supposed to be characteristic of muscle cells. Long, tapering mononuclear, or multinuclear giant cells were abundant. As a result, the section appeared in some places like undifferentiated striated muscle, or like smooth muscle, and in other places like regenerating muscle.

In the pathogenesis of this tumor, the repeated and long continued injuries to the femur must have been very important, because at the time of the fractures and the periostitis, the muscle must undoubtedly have been injured. In injuries to muscles, produced either by physical agents or by infectious processes, it is known that the muscle fibers react with some vigor, as is shown by the production of multinucleate giant-cells, and mononucleate cells resembling young muscle fibers. These elements, which are said to be derived from the normal striated muscle fiber, may be non-striated for a time, and later assume again the cross-striations. It is possible that in the present case the normal impetus to the formation of muscle cells was disturbed, and that a lawless overproduction of these elements took place. There had been for the past 27 years a chronic irritation of the surrounding muscles, emanating, as the specimen showed, from an ununited fracture.

SUMMARY

In a man, age 48, a rhabdomyosarcoma appeared at the site of an ununited fracture combined with periostitis.

The writer wishes to express his gratitude to Dr. James Ewing for his kindly criticisms and suggestions, and to thank Dr. Wm. B. Coley for permission to publish this case.

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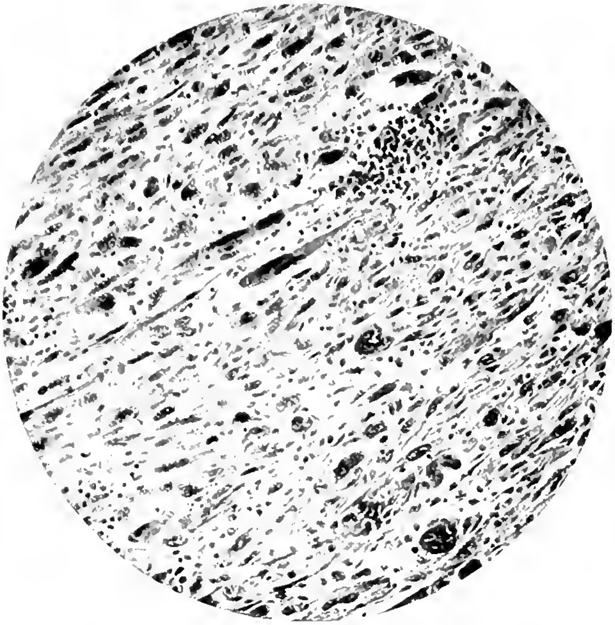
PLATE 1

Fig. 1. Large Acidophile Cells Resembling Plain Muscle. For size, compare with leucocytes present.

Fig. 2. Very Large and Long Cell, Resembling Muscle Fiber. Higher magnification: compare size with leucocytes present.



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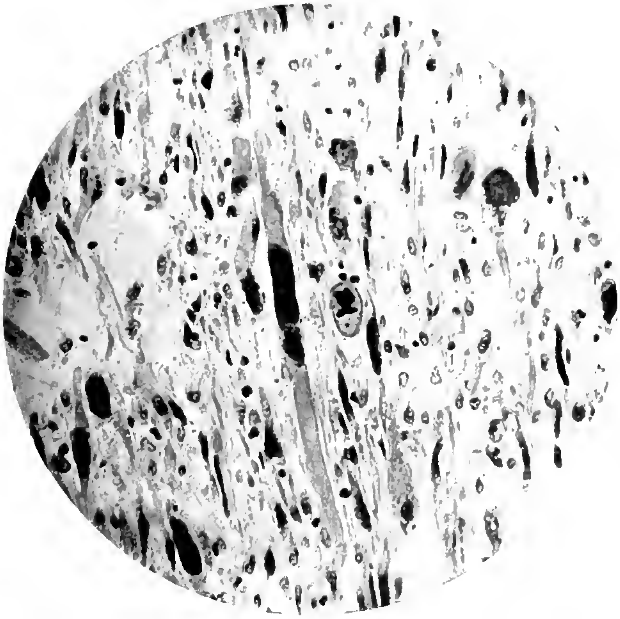

2

COMPARATIVE PATHOLOGY OF CANCER OF THE STOMACH WITH PARTICULAR REFERENCE TO THE PRIMARY SPONTANEOUS MALIGNANT TUMORS OF THE ALIMENTARY CANAL IN MICE[1]

STUDIES ON THE INCIDENCE AND INHERITABILITY OF SPONTANEOUS TUMORS IN MICE

Eleventh Communication

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GASTRIC CARCINOMA IN SPECIES OTHER THAN THE MOUSE

One of the most striking facts in the comparative pathology of cancer is the unique position of man in respect to his susceptibility to cancer of the stomach. While gastric cancer leads all other carcinomas as a cause of death in the human species, it is one of the rarest neoplasms in all other species. Thus among dogs, in which new growths are extremely common, perhaps exceeding in frequency the occurrence of neoplasms in man, cancer of the stomach is all but unknown. Fröhner (1) states that among 70,000 sick dogs there was not a single case of cancer of the stomach, although ulcers of this organ seem to occur frequently in the dog. The entire alimentary tract of all the lower animals seems to be comparatively free from cancer, if we except anal tumors in dogs (2). In his extensive review of the literature, Caspar (3) could not find a single case of cancer of the pharynx or esophagus in any species, although Cadiot (4) has described a case of carcinoma of the esophagus in a dog.

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[1] Presented before the American Association for Cancer Research, New York, April 5, 1917.

The nearest thing to an exception to the statement that cancer of the stomach is extremely rare in all animals but man, is furnished by the bovines, in which squamous cell carcinoma of the rumen is by no means infrequent, for of 78 cases of cancer in bovines collected by Sticker (5) in his extensive review, 6 were found in this location. However, the rumen is properly but a dilatation of the esophagus, and not a true stomach. No case of carcinoma has ever been described in the true stomach of a bovine, so far as we can find in the literature. A similar state of affairs exists in the horse, for of 322 cases of primary carcinoma in horses, Sticker found 8 in the "stomach," but these seem to have been chiefly, if not entirely, squamous cell carcinomas of the cardia.

Sticker could find no recorded case of cancer of the stomach in sheep, swine, or cats, in all of which cancer has been observed in other locations.

Indeed, so few are the recorded cases of gastric carcinoma observed in any species of animals, beyond those of the rumen of the cow and the cardia of the horse, that they may be described in a brief space. They are so scattered through the literature, many in publications difficult of access, that it is quite impossible to hope to locate them all, but we have found the following reports, which it may be worth while to recapitulate here as a nucleus for further additions by subsequent writers.

Dog. But two apparently authentic cases of spontaneous carcinoma of the stomach have been found,[2] although in his review in 1902 Sticker collected 765 cases of primary carcinoma of other tissues in dogs. This author questions the validity of Eberlein's (6) case, but the report seems to indicate its correctness. This was a cylindrical cell carcinoma with well-developed stroma, and produced metastases in the lungs and liver. The second case was observed, but not fully reported, by Magnusson (7). In discussing the occurrence of tumors of the heart in domestic animals, he mentions the case of a male dog with a carcinoma of the pylorus, which had metastasized into numerous organs, including the heart, but he gives no other details. Cases of sarcoma of the stomach have been reported in dogs (see Sticker and Caspar), but the descriptions commonly leave some doubt as to their character and origin (8).

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[2] Hemmeter (42) says that after producing an experimental ulcer of the stomach in a dog and keeping up a high acidity, "at the autopsy a cancer of a stomach was found near the pylorus." He also describes a dog presenting an adenocarcinoma at the edge of an experimental gastric ulcer; it "reached the size of a walnut, and two metastases were found in the omentum." There are no further details concerning the character of these growths, which, if cancers, were experimentally produced rather than spontaneous.

Birds. In their review of the spontaneous tumors of birds, Joest and Ernesti (9) reported about 160 cases, of which three were cylindrical cell carcinomas of the stomach of fowls (Weiskopf, Schöppler, Zanini), as well as a much larger number in the intestine. The case of Weiskopf may be properly excluded, since there seems to be no evidence that the primary growth was in the stomach. Schöppler (10) also quoted two other cases of carcinoma of the stomach, found among 37 malignant tumors in fowls by Fujinami (11). Wernicke (12) mentioned, without giving details, still another case of carcinoma of the stomach in a chicken, described by Sticker (13). A spindle cell sarcoma of the glandular proventriculus of a male chicken, with metastases in the liver, lung, and heart, has been reported by Joest and Ernesti (9).

Bovines. In 305 cases of primary tumors of bovines observed in the slaughter house at Glasgow, Trotter (14) found no less than 25 in the rumen, of which all were squamous epitheliomas except one "scirrhous cancer;" many originated in ulcers and some in papillomata. Although there were ten malignant tumors in this series, arising in the intestines, there was no case of cancer of the true stomach. Among twenty-six cases of malignant tumors in cows, Murray (15) found 4 squamous cell carcinomas of the rumen, and the six cases collected by Sticker have been mentioned previously. We have found no case of carcinoma of the true stomach of the cow in the literature, but Magnusson (7) mentioned a case of round cell sarcoma of the "Labmagenwand" of the cow with secondaries in the heart and elsewhere, which was reported by Gänsehals (16). Caspar (3) in his review (1896), referred to several cases of "panzerartiges

Sarkom" arising in the wall of the cardia, as well as a round cell sarcoma of the stomach, but the correctness of the diagnosis of sarcomas observed so many years ago is open to question

Horses. In the eight cases of gastric carcinoma mentioned by Sticker, there is too little description to fix the origin or character of all. He described his own two cases as being respectively a "Pflaster-zell" carcinoma and a large round cell carcinoma. M'Fadyean's three cases (7), included by Sticker, were all very malignant squamous epitheliomas of the cardiac end of the stomach; one of these was inoculated into a horse without success. Apparently in the horse, as in the cow, it is the squamous cell lining that is the chief, if not the sole site of malignant degeneration. The three cases quoted by Wolff (18) are: (1) squamous cell carcinoma of the cardia, with liver metastases (Durbeck), (2) ulcerative scirrhus with metastases in the liver and peritoneum (Hillbrand); (3) squamous cell carcinoma of the cardia, at the site of ulcers produced by "Bremsenlarve" (Petit). Wolff says that sarcoma is more frequent than carcinoma, of which the commonest type is an "Ulcus carcinomatodes scirrhosum am Pansen."

Rats. Spontaneous primary tumors of the stomach of rats must be most infrequent, for among 100,000 rats examined by McCoy (19) in his plague work, in which there were found 103 tumors, there was not a single case of neoplasm anywhere in the gastrointestinal tract. The further series reported by Woolley and Wherry (20) also contained none. An interesting exception is furnished by Fibiger (21), who found in rats infected with nematodes, numerous instances of papillary carcinoma-like growths. In 19 such growths, produced by experimental infection, 4 showed minute, microscopic metastases, and 2 others seemed to be of definitely malignant structure. These were all squamous cell growths, arising from chronic inflammation in the squamous epithelial lining of the cardia of the rats, and hence more analogous to the epitheliomas of the rumen of cows or the esophageal part of the stomach of horses. Apparently this structure reacts readily by proliferation when irritated, for Bullock and Rohdenburg (22) produced polypoid epithelial hyperplasia by introducing woolen balls soaked with chemical irritants into the stomach of rats. Adenomatous hyperplasia of the pyloric mucosa has also been observed frequently in rabbits fed lanolin for long periods by Kon (23).

In spite of the great number of albino and other domesticated rats examined in laboratories, we have found no recorded case of a spontaneous neoplasm of the stomach in these animals, and there were none among the 171 cases of spontaneous rat tumors collected or observed by Bullock and Rohdenburg (24). That this organ is at any rate, not essentially refractory to the growth of transplanted tumors, was shown by Citron (25), who obtained 25 per cent of successful inoculations of rat sarcoma in the stomach wall.

Rabbit. Neoplasms of any kind are extremely uncommon in rabbits,[3] but Schmorl (26) says:

> Twice I have found rabbits with carcinomatous tumors. In one animal it was a small carcinoma in the vicinity of the cardia, in the other a nodule in the lung with the structure of a carcinoma simplex.

We can find no further description of either of these rare cases.

Wild animals. Wild animals rarely have neoplasms of the stomach. Fox (27), however, has described a squamous cell papillary tumor arising in the pylorus of a male red kangaroo (*Macropus rufus*), with metastases in the liver, spleen, and kidneys. Renshaw (28) has reported, in the greater curvature of the stomach of a rhinoceros (about 30 years old), a triangular tumor, with the apex directed toward the pylorus, the surface flat and ulcerated; some nodules were present in the vicinity of the tumor; "the microscope showed that the tumor resembled carcinoma of man." Koch (29) reports observing a large fibromyoma ("kindskopfgross") in the stomach of a deer, arising in the rumen, and with an ulcerated, necrotic center. A fibromyoma from the stomach of a leopard was also found by Williams (30) in the collection of the Glasgow Hunterian museum, and a similar tumor has been described in the stomach of a python by Petit and Vaillant (31), which is the only case of stomach tumor of any kind in a cold blooded animal that we can find, except the very incomplete information contained in a statement made by Kantorowicz (32) who, in a discussion of another case of cancer, said that he had seen a pyloric carcinoma, with multiple liver metastases, in a three year old "Box."[4] Williams (2) quotes the case, reported by Harrison, of a male lion, sixteen and a half years old, with a columnar cell carcinoma of the ileum above the ileocecal valve. Fox has also described an adenocarcinoma of the intestine with many metastases in a male dasyure.

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[3] Fütterer (43) describes the experimental production of gastric ulcers in rabbits, and states that in one case there occurred a glandular hyperplasia resembling an adenocarcinoma. He is careful to state that he does not claim that this is a true carcinoma, although he seems impressed with the similarity of this growth with human cancer; but neither the description nor illustrations are convincing.

Of particular interest, in view of the rarity of tumors in primates, is the report by the same author (33) of a diffuse adenoma of the stomach in a male Hamadryas baboon, the only neoplasm found by him in autopsies on 381 primates. A definite case of primary carcinoma of the stomach in a primate, however, has been reported by Schmey (34), occurring in a female long tailed monkey (*Cercopithecus rufo viridis*), aged eight to ten years. It involved the stomach so diffusely that the point of origin could not be determined, infiltrated through to the serosa in many places, and produced extensive lymph node metastases. The tumor was a small, polymorphous cell medullary carcinoma, of the solid type (without tubule formation), and did not differ in any essential particular from human gastric cancer.

GASTRIC CARCINOMA AND SARCOMA IN THE MOUSE

In spite of the enormous number of mice autopsied in laboratories, and the frequency of cancer in these animals, we can find but four reported cases of primary neoplasm in the stomach of this species. These cases are as follows:

1. Murray (35) has reported a case, which is so typical of all the others, that we quote his description in full:

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[4] Presumably this is the teleostean fish, *Box vulgaris* (Cuvier).

In performing the autopsy of an old male mouse the spleen was seen to be adherent to the greater curvature of the stomach. On opening the stomach along the lesser curvature, a small ulcer was discovered with thickened edges at the point of adhesion to the spleen. The normal mucous membrane of the stomach in the mouse consists of two parts sharply marked off from each other. The cardiac two-thirds is lined by stratified squamous epithelium directly continuous with that of the oesophagus, and comparable to the rumen of herbivora. The pyloric portion, much smaller in extent, forming one-third of the whole organ, is lined by a glandular epithelium similar to that of other mammals. The ulcer under discussion is situated in the squamous-celled portion immediately adjacent to the glandular part. The squamous-celled alveoli extend laterally under the adjacent glandular and normal squamous mucous membranes. They have infiltrated and expanded the circular muscular coat, extended through the longitudinal layer, and expand under the peritoneal covering into a small cyst. Isolated alveoli lie external to the deeper layer of this flattened cyst between the adjoining lobes of liver and pancreas.

2. In concluding the above report Murray merely mentioned a second case, as follows: "An earlier stage of the same condition was discovered at autopsy in an aged female mouse, which presented a carcinoma of the right axillary mamma." He published a photograph of the gross appearance of the stomach, and merely added that it resembled closely the more advanced case.

3. Little and Tyzzer (36), in a paper on the "Inheritance of Susceptibility to a Transplantable Tumor," described a case as follows:

A carcinoma of the stomach, which originated in a laboratory stock of wild mice, grew when implanted in four of eleven, but on the second transfer failed to grow in twelve mice of the same stock.

In a letter Dr. Tyzzer has given us further information concerning this case, which he kindly permits us to publish. He states that it was in a female wild mouse, and adds:

The mouse in which the original tumor occurred was inoculated with a tumor about a year and seven months previously. This apparently failed to develop, as subsequent negative observations indicated. It was killed on account of the appearance of a rounded tumor beneath the skin. This had the general appearance of a sebaceous cyst except for a mass of pinkish tissue in one portion of the wall. Axillary and inguinal lymph nodes were not enlarged. No metastases were apparent in the lungs. There was a mass of tissue infiltrating the greater curvature of the stomach, measuring 3 mm. in thickness and somewhat over 1 cm. in extent. The left ovary was replaced by a mass of similar tissue. There were nodules distributed along the uterus and numerous small masses having the distribution of the post-peritoneal lymph nodes. These latter metastatic nodules were used for the transplantations. The transplants appeared as rounded nodules but on section were found to contain a collection of fluid which turned litmus paper blue.

The histological examination of the subcutaneous nodule showed a tumor the epithelium of which corresponded with that of other mammary tumors of the mouse. There was, however, a tendency of the epithelium to undergo a sort of pseudo-cornification; that is, there were foci and layers of swollen red-staining cells. The tumor of the stomach was composed of epithelium having a distinct epidermoid tendency in its differentiation. The squamous epithelium of the cardiac portion of the stomach showed a very marked papillary change and the tumor epithelium resembled this so closely that it appeared to me most probable that it had arisen in this portion of the stomach. The tumor showed marked invasive and metastasizing qualities so that it had replaced the muscular layers of the stomach and it was found widely distributed throughout the lymph nodes of the abdominal cavity.

4. Itami (37) has reported a case from the Crocker Fund laboratory, occurring in a white female of unknown age, from which two adenocarcinomas had been extirpated, without recurrence, about twelve weeks before it was killed because of progressive loss of weight. The squamous portion of the stomach was diffusely thickened by a squamous cell carcinoma, which did not involve the pyloric portion. There were no metastases. Forty-eight mice were inoculated from the cancer, without any takes.

To these four cases we wish to add four more, which represent all the instances found in 16,500 autopsies on mice, nearly all dying a natural death, all without experimental manipulation or interference with their natural development, mostly at advanced age, and in a stock which has yielded approximately 2000 mice with tumors of other sorts. Every suspicious spot in any organ has been examined microscopically, and many inflammatory lesions of the stomach thus excluded.

5. A male mouse (no. 5802), two years old. Near the pyloric portion of the stomach is a mass about 15 by 15 by 12 mm., occupying the wall of the stomach, and growing out towards the pancreas, which it does not seem to invade. The external surface of the stomach is marked out by irregular nodules. The internal surface is ulcerated, folded, slightly papillomatous, and somewhat discolored in the cardiac portion, both anterior and posterior surfaces being involved, but not the pylorus. The wall varies from 3 to 5 mm. in thickness.

In the mesentery is a roughly spherical white mass about 11 or 12 mm. in diameter, not adherent or infiltrating. The central portion is softened, and the periphery is nodular. No nodules are found elsewhere. The spleen and liver are large and full of amyloid; the kidneys show but little change; the lungs exhibit no gross changes and especially no metastases.

Microscopically, the primary tumor is seen to be composed of masses of squamous epithelium, forming typical cancer nests with hornified centers (fig. 1). The growth extends to the junction of the squamous and cylindrical epithelial portion of the stomach, and spreads freely from the former under the latter, which is not ulcerated. For the most part, the cancer tissue is necrotic in the center, but is not much ulcerated except at one point where there is ulceration through to the serosa. The cancer replaces all the coats of the stomach, and protrudes into granulation tissue and adhesions attached to the serosa. The pancreas is firmly adherent to the stomach over the tumor, and there is a slight invasion of the superficial portion of the pancreas. Mitotic figures are abundant in the peripheral parts of the growth.

The mesenteric nodule consists of a central necrotic mass, containing many large bacteria without cellular reaction (post

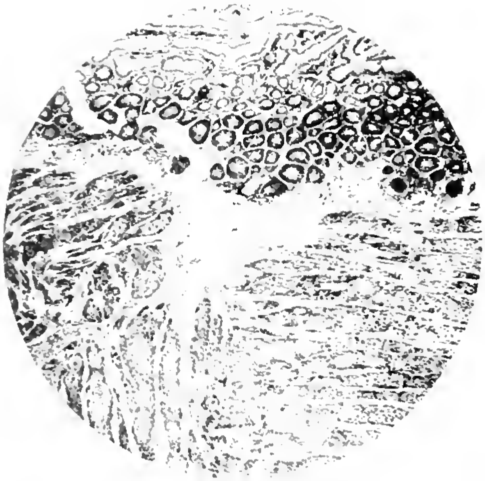

FIG. 1 Photograph showing the typical squamous cell growth infiltrating beneath the glandular mucosa of the pyloric portion of the stomach. × 70. (Case 5, our no. 5802.)

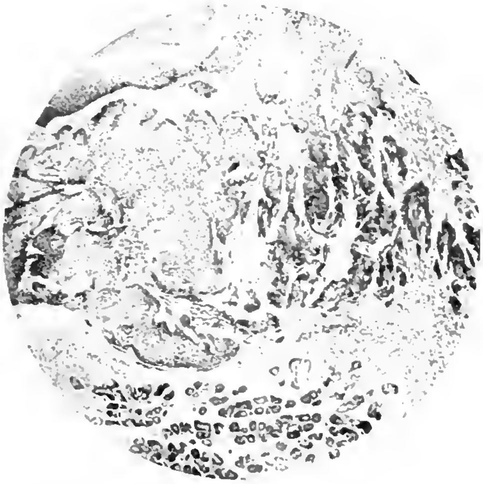

FIG. 2. Showing the growth of cancer as epithelium from the squamous cell lining of the stomach, through the muscularis, into adhesions binding the pancreas to the stomach. × 60. (Case 6, our no. 7851.)

mortem invasion), with a narrow peripheral zone of plugs of squamous cells. There is a relatively thick capsule of young connective tissue infiltrated with round cells and, in some places, with collections of polymorphonuclear leucocytes; it is invaded freely by the cancer cells, which show very few mitotic figures. No recognizable lymph node tissue is found. The spleen contains much amyloid, the liver less, and the kidneys very little; otherwise these organs show no noteworthy changes. The lungs are normal and no tumor emboli are found.

Diagnosis. Squamous cell carcinoma of the cardiac portion of the stomach, with metastasis into mesentery. General amyloidosis.

6. This was also a male (no. 7851), twenty-five months old, and the uncle of mouse 5802. The cardiac end of the stomach is diffusely thickened and indurated with coarse lobulations visible externally, its internal surface being slightly ulcerated and thrown into small folds. The ulcer measures 16 by 12 mm. and is 8 mm. thick at the thickest part. The rest of the stomach is full of pus. No parasites are found in the stomach. The liver is small, hyperemic, and adherent to the stomach. The spleen is stiff with amyloid but not much enlarged; the kidneys are small and hard; the lungs bloody, but without tumor nodules. No metastasis could be found.

Microscopically, the growth is limited to the cardiac portion of the stomach, where the entire squamous epithelial surface is underlaid by masses of typical squamous cell carcinoma nests with extensive hornification. The growth has almost completely replaced the muscular layers and infiltrated the serosa (fig. 2). The adhesions between the liver and stomach are infiltrated with cancer nests, but the liver itself is not invaded. Mitotic figures are scanty. In one place, a large pus sac is found, surrounded by a layer of stratified epithelium on a granulation tissue base. The spleen and liver show advanced amyloidosis, and the lungs are edematous. No metastases are found in any place.

Diagnosis. Squamous cell carcinoma of the cardiac portion of the stomach. General amyloidosis.

7. Since the preceding two cases were reported to the Association for Cancer Research, we have obtained a third cancer of the stomach quite similar to them. This occurred in a female mouse, (no. 16440), aged about 27 months. The animal was second cousin to female no. 12614, reported in this paper.

The stomach is greatly enlarged, measuring about 25 by 15 by 10 mm. At the junction of the cardiac and pyloric portions of the stomach is a thickened mass of tissue, extending nearly around the stomach, only about 3 mm. of the dorsal side being of normal thickness. This thickened portion at its widest point on the ventral side of the stomach is about 15 mm.; on the dorsal side it is 3 to 5 mm. This thickening does not extend to the pylorus, the pylorus and the pyloric portion of the stomach being quite normal in appearance and thickness for a distance of 3 to 5 mm. The surface of the tumor is slightly hemorrhagic, and although somewhat lobulated it is smoother than the other tumors studied. The consistence is quite firm and the thickness is uniformly 2 to 3 mm The inner surface appears only slightly ulcerated. On the ventral aspect there is a hard white nodule, 3 mm. in diameter, in the omentum. There is a similar larger white nodule in the root of the mesentery, which is about 5 mm. in diameter.

There was nothing noteworthy elsewhere. The lymph nodes were very slightly enlarged; the spleen was much enlarged. There was no indication of metastases elsewhere.

Diagnosis: Carcinoma of stomach with metastases in regional nodes.

Microscopically, both the squamous and glandular surfaces are underlaid by a most diffuse and extensive squamous cell growth, involving all structures from the serosa to the lumen, and almost completely replacing the muscularis. Cancer nests are occasionally found, also, among the tubules of the mucosa, but there is here very little ulceration. There is more ulceration in the squamous portion of the stomach. Hornification is often seen, but is not prominent; mitotic figures are rare.

The nodule in the omentum consists of a solid mass of cancer tissue without evidence of any pre-existing lymphatic tissue.

Here the growth consists of a solid mass of densely packed plugs with much hornification. There is a similar microscopic nodule adjacent to the larger one.

The lymph node at the root of the mesentery shows a nodule similar to that in the omentum, although with more hornification; some of the lymphatic tissue still remains.

The liver and spleen show extensive amyloid deposit, especially the former. No metastatic growths are found in either of these organs, or in the lungs.

It will be noted that all these seven cases involve the cardiac portion of the stomach, and are squamous cell cancers. This brings them into the same category with the stomach cancer of the cow and horse, which involve almost or quite exclusively the squamous portion or rumen. Especial interest, therefore, attaches to the following case, which concerns a cancer arising from the glandular, secretory pyloric portion of the stomach.

8. A male mouse, aged two years and seven months (no. 14580). In the lower part of the pyloric portion of the stomach the wall is greatly thickened, practically occluding the lumen, and forming a solid mass about 8 mm. in diameter. The wall of the stomach here is 1.5 to 2 mm. thick, the external surface being smooth. This is not adherent to the adjacent structures, except the pancreas, and is covered by a smooth serosa. A few of the adjacent peripancreatic lymph nodes are slightly enlarged. In the stomach is a hair ball, and the pyloric mucosa is thrown up into longitudinal folds and superficially ulcerated. The lower part of the intestines contains a bloody fluid. The liver is pale and slightly enlarged, but not involved in the tumor. The spleen is enlarged to about four times its normal size. There are no secondary nodules to be found in any other viscera, and these show no marked changes, except that the seminal vesicles are atrophied and the left cardiac ventricle is hypertrophied.

Microscopically (fig. 3), the growth involves exclusively the glandular, pyloric portion of the stomach. Passing insensibly from the uninvolved portion of the mucosa, which shows much evidence of chronic inflammatory changes, is a growth of atypical tubular structure which infiltrates freely through the muscularis, and even invades the adherent pancreas. Very few mitotic nuclei are seen. The tubules are generally distended and exhibit a low, flattened epithelial lining, much resembling a "colloid cancer" of the stomach, except that the lumina of the tubules contain no mucin or other stainable material. There is a moderate round cell infiltration in the growth, and considerable stroma formation, with much fibrous thickening in the serosa where it is adherent to the pancreas. This fibrous tissue is extensively infiltrated by tumor tissue with collapsed tubules, and simple cords of cells.

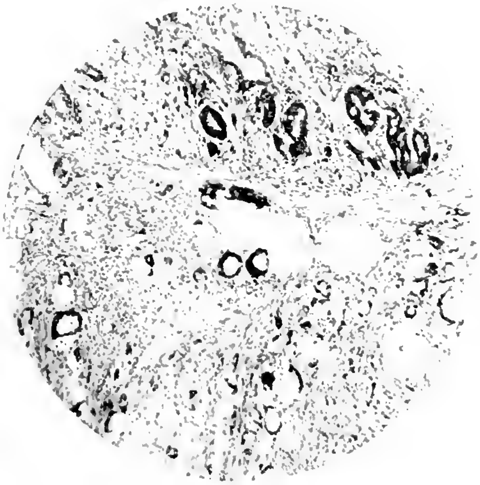

FIG. 3. Tubular carcinoma of pylorus infiltrating the submucous and muscular layers, with dilatation and pressure atrophy in many of the tubules. The glandular mucosa is seen in the upper part of the section, exhibiting round cell infiltration and fibrosis, presumably resulting from the presence of a hair ball. × 90. (Case 8, our no. 14580.)

This growth resembles very closely in its appearance the tubular carcinomas of the stomach seen in man.

Section of two of the regional lymph nodes shows them to contain numerous tubules, some isolated and others in clusters, with a little fibrosis about the infiltrating tumor tissue.

No metastases are found in the lung or liver.

Diagnosis. Primary tubular carcinoma of the stomach, with metastasis into regional lymph nodes. Hair ball in the stomach, with chronic gastritis.

Recapitulation. Of the eight cases of carcinoma of the stomach of mice now recorded, seven were squamous cell carcinomas involving solely the cardiac portion, and only one corresponds to the tubular, cylindrical cell type of cancer found in man. Four occurred in females, and four (including three of our four) were in males. Four showed metastases into the regional lymph nodes, one having in addition an ovarian metastasis. Two also had primary mammary gland carcinoma. Inoculation was tried with only two, and in one of these a few takes were obtained in the first generation, which could not be carried to a second generation.

We have observed no instance of secondary carcinomatous growths in the stomach among approximately 2000 mice with spontaneous tumors.

We have found no recorded case of sarcoma involving the stomach in the mouse, although sarcoma is by no means a rare growth. Thus, in a recent report (38), we collected from the literature 17 cases of sarcoma in mice, and recorded in addition 87 cases observed in 12,000 autopsies on the Slye stock, but none of these sarcomas arose in the stomach. Since then we have observed a case that resembles very closely a primary sarcoma of the stomach. The record is as follows:

Female (no. 12614), age twenty months. Beginning at the duodenum, and spreading up into the cardiac portion of the stomach, is a diffuse growth of tissue forming a mass about 20 by 18 mm. in diameter, and up to 12.5 mm. thick. It is pink and fleshy, covered by intact serosa, and without exudate or necrosis on either serous or mucous surface. The external surface is coarsely lobulated and the growth is mostly posterior, leaving the anterior surface of the stomach relatively free. This infiltration also surrounds the common bile duct and follows it up into the hilum of the liver. In the bile duct is a tape worm, but the typical extensive suppurative cholangitis and hepatitis commonly found in these cases, is not present. The liver does, however, contain several small nodules of tissue resembling that of the tumor. The extreme cardiac end of the stomach is shrivelled and empty. A mass in the mesentery, fitting over the stomach like a cap, but not adherent to it, measures 20 by 12 mm. across and 8 to 10 mm. thick. From this a few strands run to a smaller mass in the mesentery. The diaphragm is thickened and on the right side shows a mass of neoplastic tissue. No nodules were found in the lungs.

The spleen, ovaries, and the lymph nodes in general, were not noticeably enlarged.

Microscopic appearances. Infiltrating the stomach wall diffusely, and widely separating the mucosa from the serosa, is a uniform growth of small, nearly spherical cells (figs. 4 and 5). These are somewhat larger than lymphocytes, less spherical, and have generally much less intensely staining nuclei. They are not uniform in size, although there are no very large cells and no multinucleated forms The cytoplasm is scanty, and stains rather deeply, resembling somewhat the cytoplasm of plasma cells. The nuclei vary from those with chromatin as dense as that of a lymphocyte, to vesicular and occasionally lobulated forms; generally the chromatin occurs in closely packed fine granules. No mitotic nuclei are seen, but there is frequent amitotic division. The growth is entirely uniform throughout, and infiltrates and destroys the muscular tissue freely, so that in most of its extent no muscle cells remain. There is no inflammatory reaction of any kind, and the mucosa is everywhere intact. The serosa is infiltrated, without any adhesion or fibrous proliferation, but there is very little invasion of the mucosa. There are a few blood-vessels, but there is no necrosis or other retrogressive change. Mitoses are not found. The growth involves both the pyloric and cardiac portions of the stomach.

A similar tissue is found infiltrating the hilum of the liver along the bile ducts, and in places distinct nodules have formed in the liver. There is some suppuration in the larger bile ducts. Beyond invasion by the tumor, the liver shows little change, and does not exhibit the perivascular infiltration characteristic of leukemia and pseudoleukemia.

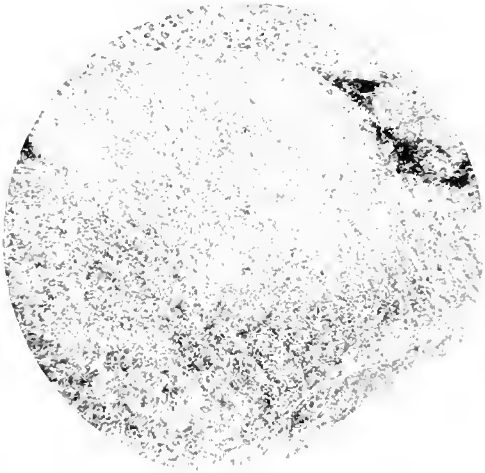

FIG. 4. Very low power view to indicate the extent of infiltration by sarcoma tissue of the stomach wall, which is shown in the entire thickness in one of the thinnest parts of the invaded wall. Above, the glandular mucosa can be seen, and the dark patches of lymphoid cells serve to indicate the relative size and nuclear density of the neoplastic tissue. × 45. (Our no. 12614.)

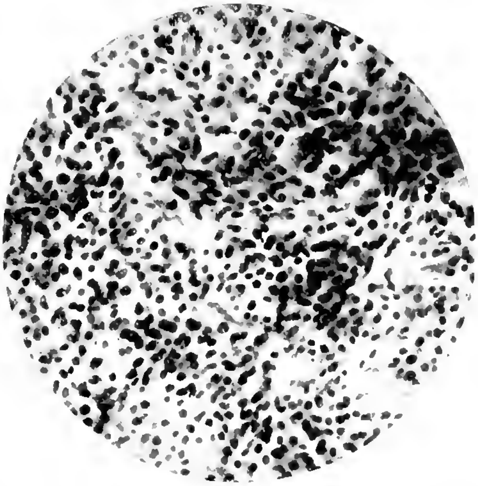

FIG. 5. High power view of the tissue in the stomach wall, reproduced in figure 4, to show the cell structure. The entire neoplastic tissue in this case is of similar structure. × 150.

The kidneys and lungs likewise show no evidence of lymphoid deposits, although the latter does contain a few small nodules of tissue, apparently metastatic growths of hematogenous origin, resembling, in location and character, typical tumor metastases rather than leucocytic infiltration or lymphoid hyperplasia.

The spleen contains much amyloid, and shows also irregular patches of tissue similar to that in the stomach and liver; this contrasts sharply with the lymphoid tissue of the spleen, the cells being paler, slightly larger, and with more cytoplasm. There are no definite nodules of neoplastic tissue in the spleen.

The diaphragm is infiltrated with the same type of tissue, which destroys the muscle, and in one place forms a distinct nodule.

A huge mass of mesenteric lymph nodes, is present. Two of these, measuring respectively 7 and 9 mm. in diameter, are so infiltrated with tissue of the same character that almost no lymphoid tissue can be found.

The blood-vessels show no excess of leucocytes in the circulating blood.

We are by no means sure of the correct diagnosis in this case. In nearly all respects it corresponds to a small round cell sarcoma, arising in the wall of the stomach and producing the type of local extension usual in lymphosarcomas; very similar lymphosarcomas, furthermore, have been described in the stomach and intestine of man. The only grounds for reluctance in diagnosing this as a primary lymphosarcoma of the stomach are, first, the general doubt as to the real character of all growths of this lymphosarcomatous character, especially emphasized by their unsolved relation to the pseudoleukemias. Second, we find in the spleen a diffuse growth of cells similar to those of the tumor, which do not occur in distinct nodules as typical secondary tumors should, but rather resemble the results of a hyperplastic change in spleen cells. We have seen so many instances of tumor-like proliferations in the liver, associated with diffuse growth of similar cells in the spleen, and apparently presenting in different cases various stages between conditions that seem almost certainly inflammatory or granulomatous, to others not certainly distinguishable from true sarcoma, that we have become very doubtful of the advisability of making positive diagnoses of sarcoma in related conditions. They sometimes recall certain of the cases of Gaucher's disease and other splenomegalic conditions. Nevertheless, in most respects this case seems to be as typical a sarcoma as any gastrointestinal lymphosarcoma described in man, and we are quite unprepared to say that it is not a sarcoma. The gross findings are even more characteristic of sarcoma than the microscopic, but it is impossible to say whether the growth arose primarily in the stomach or in the mesenteric lymph nodes.

INTESTINAL CARCINOMA IN THE MOUSE

Although intestinal cancer is not extremely rare in the lower animals, especially in birds, yet in mice it is no more common than cancer of the stomach. In the literature we have found mention made of this condition only by Murray. In the Third Scientific Report of the Imperial Cancer Research Fund (p. 71), he says that carcinoma of the intestine has been reported by Bashford, Murray, and Cramer, and by Twort. We have found no case of adenocarcinoma, similar to those described from the London laboratories, in our mice. The only intestinal carcinoma in the Slye stock occurred under the following conditions:

A male (no. 8345) twenty-seven months old, a cousin of mouse 7851, which had cancer of the stomach, developed a prolapse of the rectum and was kept under observation in the "hospital" for the six months preceding his death. About four months before death, it was noticed that the prolapsed portion of the rectum had become ulcerated. The hair became scanty, and the mouse emaciated greatly. The protruded rectum became much indurated, and somewhat ulcerated, forming a hard mass about 6 mm. in diameter at the time of death. Autopsy disclosed no other noticeable abnormalities, beyond the reduced size of the organs associated with the emaciation.

Microscopically, the mucosa lining the prolapsed part of the bowel is practically normal, but that covering the protruded portion is much altered. The mucosa and submucosa show extensive chronic inflammatory changes, and the mucous glands are much hypertrophied, many of them also being dilated to cystic proportions. Part of the surface is covered by squamous epithelium which overlies mucous glands, and, being discontinuous, is apparently of metaplastic origin, at least in part. Some areas of this squamous epithelial coat show hyperplasia with the development of downgrowths of columns of epithelium, and in one considerable area the downgrowth has assumed a distinctly malignant character (fig. 6). Here the columns of squamous epithelium spread extensively into the muscularis, and grow freely in the lymphatic channels, in places penetrating nearly to the mucosa of the internal portion of the rectum. The centers of the epithelial plugs show marked hornification, and outside them is a moderate round cell proliferation. The

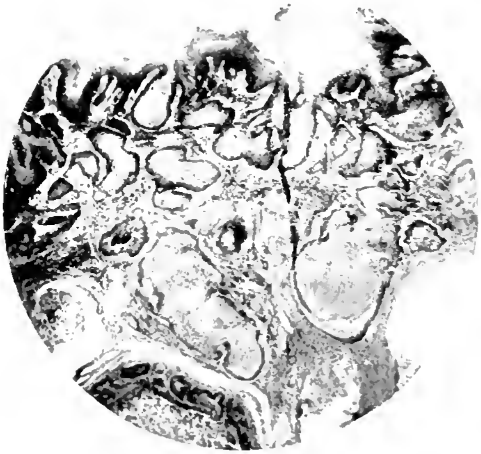

Fig. 6. Section through prolapsed rectum, showing the heavy squamous epithelial coat that covers the external surface of the bowel. From this the squamous cell plugs infiltrate through the coats of the bowel to the tubular glands of the rectum, seen in the lower left hand corner. × 60. (Our no. 8345.)

regional lymph nodes show no metastasis. There are no changes of interest in the other tissues.

Diagnosis. Early squamous cell carcinoma developing in the mucosa of a chronically inflamed, prolapsed rectum.

DISCUSSION

The demonstrated fact that the alimentary tract of all the lower animals is so rarely the seat of primary tumors, affords such a striking contrast to the frequency of malignant change in the stomach and intestines of man, that it would seem probable that herein must lie important evidence as to the etiology of cancer. It is evident that in the gastric cancer of animals, as elsewhere, chronic injury is important; witness the prevailing location of the disease in the cardiac portion or rumen, where the hard food materials are held, and where simple ulceration is common. The single case of carcinoma of the pylorus in a mouse apparently resulted from the irritation of a hair ball. Fibiger's neoplasms in the rat stomach are evidently the result of irritation rather than of specific infection. It is of interest that despite the prevalence of macroparasites in the alimentary canal of all the lower animals, neoplasms of this tract are so infrequent. We note also that in none of the eight recorded cases of cancer of the stomach in mice was there any evidence of the presence of animal parasites, and hence they are not analogous to Fibiger's observations.

The frequency of gastric carcinoma in man, naturally, is commonly attributed to the special character of his food, which differs from that of all other animals solely in that it is cooked and seasoned. Its actual materials can scarcely be held responsible, since carnivora, herbivora, and omnivora are equally free from gastric cancer. The possibility that the heat of our foods may be responsible has been an attractive hypothesis, and the evidence on this subject has been so well and so recently reviewed by Lerche (39), that further discussion is not required here. This author found the evidence very convincing that carcinoma of the stomach and esophagus is related to, and probably dependent upon, the use of hot foods and drinks. He calls particular attention to the correlation of the well-known variation in geographical distribution of cancer of the stomach with the practice of the populations in respect to hot food and drinks, for cancer of the stomach is frequent in the north temperate zone where much hot tea and coffee are consumed, and rare among tropical and sub-tropical peoples who do not use these beverages. The chief objection to this hypothesis lies in the fact that carcinoma arises most often in the pyloric region, whereas it would be expected that hot food and drink would most affect the esophageal orifice and the cardia. Lerche collects much evidence that corrosive fluids produce their chief effects along the lesser curvature and in the pyloric region, particularly the latter. On the other hand, the direct measurements of the temperature of the stomach contents by Stengel and Hopkins (41) indicate that the fundus is much more affected by changes in the temperature of the food than is the pylorus. It would seem probable, on the basis of all these observations, that the food first impinges on the lesser curvature and the pylorus, to come to rest in the fundus; if this be correct, the influence of the hot food as an irritant might be greater at the pylorus and lesser curvature than at the cardia, although the average temperature might be more modified by food in the cardia. Whenever hot foods or drinks are taken, the greater curvature and cardia would be protected by the materials already present, unless the stomach were absolutely empty, whereas the mucosa of the lesser curvature and pylorus would come in momentary contact with the hot foods undiluted.

The influence of condiments in foods might also well be of importance, in view of the profoundly irritating character of most of them, but unfortunately we have no reliable statistics as to the frequency of cancer of the stomach in such countries as Mexico, where fiery condiments are most used. However, Williams (2) quotes Jourdanet as stating that cancer is almost unknown in the hot regions of Mexico, whereas in the cool regions on the high plateaux it is frequent. This statement does not differentiate between cancer of the stomach and the other locations, unfortunately. The figures on cancer mortality in Mexico City, quoted by Hoffman (40), indicate a low cancer rate without any particular frequency of gastric cancer, but the reliability of these data is open to question.

Another possible factor, which we have not seen mentioned, is the chemical change in food that is produced in cooking. It is generally recognized that the products of incomplete combustion of organic materials have a particularly marked tendency to irritate and stimulate the skin to the point of malignant degeneration, as seen in chimney sweeps, and in paraffin, pitch, and tar workers. May it not be possible that in the cooking of foods similar products are formed, especially where the heat is high, as on the surface of baked and roasted foods? Domesticated animals, although fed on cooked foods to some extent, probably never have them constitute so large a proportion of their diet as man.

It would seem impossible to escape the conclusion that there is something about the preparation, composition, or manner of preparing food that accounts for the frequency of cancer of the stomach in man, for surely mere mechanical irritation, and acute ulceration, are at least equally conspicuous in the stomachs of the lower animals. This striking difference affords a clue for profitable investigation as to the cause of gastric cancer.

SUMMARY

A review of the literature shows that in all animals except man, carcinoma of the stomach is extremely uncommon and the recorded cases found in the lower animals are here collected and discussed briefly. In animals with a rumen, or in which the cardia is lined by squamous epithelium, carcinoma of this tissue is observed much more frequently than in the glandular gastric mucosa. Only four cases of carcinoma of the stomach of mice could be found recorded in the literature, all in the squamous cardiac portion. In 16,500 autopsies on mice dying natural deaths at all ages in this laboratory, we have found three squamous cell carcinomas of the cardia, and one tubular pyloric carcinoma; the latter apparently resulted from the presence of a hair ball in the stomach. One gastric neoplasm, resembling closely a sarcoma, was also observed. Carcinoma of the intestine is also very rare in mice, the only case observed in the Slye stock being a squamous cell carcinoma arising in the external surface of a chronically prolapsed rectum. The significance of man's susceptibility to carcinoma of the stomach is not yet known, but probably it depends on the heat of his food or the condiments employed in seasoning it or on the chemical changes produced by cooking.

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TRAUMA AND PRIMARY MOUSE TUMORS

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These investigations were begun in the confessedly slender hope of finding a stimulus, injury, or irritation, which when applied to the mammary gland of the mouse would incite neoplastic proliferation and lead to the development of true tumors. They were undertaken in the belief that the basis offered by heredity for the origin of tumors needed to be supplemented by a stimulus originating independently of heredity, whether supplied by an injury of a mechanical or chemical nature, and with or without the intervention of commonly recognized parasites. They were suggested more directly by an observation[1] concerning the latter, viz., that the common mites which infest more or less the skin surface of all adult mice were occasionally present subcutaneously, though dead and often disintegrated, in tumor mice and other old mice, while in young mice they were rare in this location. While other parasites such as lice or bedbugs are occasional and when present plainly evident to the naked eye, mites on the other hand are constant and inseparable commensals of all mice at or soon after the acquirement of a hairy coat, and require at least some magnification for their recognition. To such organisms one might naturally look first for any parasitic agent involved in the causation of tumors in mice. In the present connection, they were regarded only as possible secondary factors such as carriers of a less tangible primary agent, or as the instrument of injuries, however subtle, acting as a stimulus. An extensive and varied exploitation of these common parasites, however, furnished a body of negative data and led to the use of other forms of irritation and trauma, with results of some positive significance.

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[1] Marsh and Wülker: Zeitschrift für Krebsforschung: about March, 1916. Not seen in published form by the writer.

In most of the experiments it was necessary to use mice of mixed or unknown ancestry, since those of the more suitable inbred strains of proper age were not yet available. These miscellaneous mice contained irregularly distributed tumor strains; the controls, which therefore presented some difficulties, are discussed under the few experiments which gave results raising the question of control. Considerable detail has been omitted from the descriptions of experiments resulting negatively or equivocally. Only epithelial mammary tumors, developing after experimental procedure not involving transplantation, or arising spontaneously in the controls, are dealt with; no transplanted tumors were used. Most of the tumors were diagnosed microscopically, and in the exceptions a satisfactory gross examination left little room for error. All the mice used were females, normal unless otherwise stated.

The statement of the duration of experiments has usually been condensed into an "average survival," in days or months, which is obtained by adding together the number of days each mouse survived and dividing the result by the number of mice in the experiment at the beginning.

The first trials aimed to open the skin, in imitation of the bites and other injuries to which mice are naturally liable, and through which the mites of the skin would, though probably involuntarily, find their way beneath the skin and thus be in a position to influence the mammary epithelium.

NEEDLE SCRATCHES AND PUNCTURES

Ten normal mice, abandoned as breeders, and more than 10 months old, were scratched throughout the skin of the mammary region with fine needles set in a rubber cork. The injury just sufficed to open the skin in many places. The average survival was 85 days. Twenty similar mice were punctured many times through the skin of the left axillary and thoracic region with a very sharp dissecting needle. The mice survived an average period of 42 days. Five of them lived for three months. The result in both cases was entirely negative.

SCALPEL SLITS

The skin in the mammary regions of 33 mice similar to the above was slit in many places with a sharp scalpel point. One tumor resulted after three months.

FILE PUNCTURES

A round, slender, slightly conical and sharply pointed jeweler's file was used to open the skin. The skin in all the chief mammary regions, held in folds, was punctured in many places, the rough surface of the file serving to scrape the edges of the wound and carry in scurf, parasites, and sometimes hairs. In three experiments, a total of 82 old females past the breeding age, though the exact age was unknown, were treated in this way. Six tumors in six mice resulted between the first and fifth months. The mice survived an average period of 4.2 months.

CONTROLS

Such mice are said by breeders to yield about 5 per cent of tumors, but this refers to their careers as breeders. The mice used were those which had failed to produce tumors while breeding, the tumor mice for the breeding period having been already weeded out. They were obtained as mice 10 months of age or older. Direct controls, comprising 55 similar mice from the same sources, were employed, and but one tumor developed among these. Their average survival period was 3.9 months, or but slightly shorter than that of the experimental animals. One might regard as controls 148 similar mice which gave entirely negative results after experimental procedures of a different nature from the punctures with a file above described. At any rate, an appreciable though not striking increase of tumor incidence seems to have been attained. This was regarded as possibly related to skin parasites carried into the wound, whether or not the trauma made by the file itself played any part, and led to more definite and intensive tests of the acarines of the skin.

Seven spontaneous tumor mice were treated in a manner similar to the preceding, save that the punctures were confined to the region of the mammillae where the latter were free from tumor involvement. The mice lived an average period of only 30 days. One had developed within 14 days a few minute adenomata at the site of the punctures; the other 6 were negative. The short expectation of life among tumor mice minimizes their value in experiments such as these.

The procedure involving file punctures was varied somewhat by first scraping vigorously a small area of the skin with a file and then puncturing the skin, in the thoracic region only, with the same file. Twenty old female mice which had finished breeding were used. They survived only about 60 days, and the result was entirely negative.

IMPLANTS OF SKIN WITH HAIR STUBBLE

The skin of the ventral surface of two old females past the breeding age was shorn with scissors of most of the hair, leaving a short stubble, practically all the mites remaining on the skin. This area of skin was then removed and cut into many small bits, which were then implanted subcutaneously in the right axillae of 26 similar mice. A trochar was used for making the implantation and the procedure was carried out in the hot room at about 37°C. Result negative.

SKIN SCRAPINGS INJECTED

The hair on the ventral surface of 3 old abandoned female breeders was clipped as in the preceding experiment, and the stubble then scraped vigorously with a razor blade. The scrapings were suspended in salt solution and injected subcutaneously in the left axillae of 30 similar mice. Result negative.

IMPLANTS OF SKIN SCRAPINGS

The ventral hair of 18 female breeders over one year old was clipped to a short stubble, and the skin was then scraped with razor blades. The scrapings appeared as a fine fuzz in which varying numbers of living and dead mites were to be seen with a lens. The scrapings from each mouse were then implanted under the skin in the left axilla of the same mouse. The average survival was nearly four months. Result negative; the scrapings were finally completely absorbed.

The experiment was repeated with 16 older mice (16 months and over). The scrapings did not, however, contain large numbers of parasites. One mammary adenoma resulted after several months.

Twenty colored and white virgin female mice, five months old, were treated as in the preceding experiment. The scrapings from these young mice contained few mites. Six of the mice were killed after 14 months, at the end of the experiment. The average survival was 8.8 months. Result negative.

Nine spontaneous tumor mice, each bearing one tumor, were implanted subcutaneously, at a point distant from the tumor, with their own skin scrapings, in a manner similar to that of the two preceding experiments. These scrapings, being from old mice, contained many living mites and a far greater number of dead ones. The subcutaneous tissues were broken up with a tracer, and the scrapings somewhat widely distributed about the point of insertion through a small slit in the skin. The mice were kept paired with males for breeding, both before and after the implantations, but produced very few young. Five survived 15 to 100 days and were negative. The remaining 4 lived 17 to 94 days and all had mammary tumors, in addition to the spontaneous growths present when the implants were made. In 3 of these, the additional tumors were in the implanted region; one had two tumors, while the two others each had one, in this area. Of these additional growths, however, two arose within 6 days after the implant, one within 17 days, and two within 3 months.

Five spontaneous tumor mice were similarly implanted with skin scrapings and caged separately without males. All died between the fifth and forty-seventh day. None had visible tumors. At autopsy, two were negative after 5 and 18 days respectively. One showed a hypertrophied mammary gland at the site of implant, after 7 days; a second had several microscopic adenomata in the implanted area after 41 days. The third developed an abscess in this region, which had almost completely evacuated at death, after 47 days; closely adjoining it was a minute white cyst, the wall of which contained a definite sharply circumscribed adenoma, the smallest independent mammary neoplasm yet seen by the writer. It was discovered only in microscopic sections, was probably disc shaped with very thin edges, and had a diameter of something over 1 mm. The extreme thickness appearing in the few sections made was 0 57 mm The adenoma contained a number of minute cysts, and was itself held in the wall of a connective tissue cyst filled with fluid, and over 3 mm. in diameter after embedding.

Ten females 3 months old were implanted subcutaneously in the left axilla with skin scrapings obtained from 6 spontaneous tumor mice. In old mice such an implant is usually not followed by a notable reaction; in these young mice however, a distinct local reaction promptly occurs, consisting of an inflammatory tumor containing a milky fluid which apparently is not always purulent. These tumors subside slowly, a slight fibrosis remaining after several weeks or months. The mice were kept virgin and none died during the first 13 months of the experiment. Seventeen months after the implant 5 survive, at the age of 20 months. Three of the 10 have developed tumors, 1 after 7 months, the other 2 after 15 months. None of these tumors was in the left axilla.

Such local irritation as is caused by the reaction cyst or abscess which always forms at the site of implant in young mice, evidently has no tendency to determine the site of true tumors appearing later; nor has any tendency been observed for the occasional spontaneous abscesses in mice to be followed by, or to fix the location of, future tumors.

Twenty virgin mice 6 months old were similarly treated, save that the skin scrapings were obtained from 20 old normal female mice. The usual local reaction followed the implantations, but the ultimate result was substantially negative, 1 mouse developing, after 4 months, 2 tumors at a distance from the implant.

It is thus seen that the implantation of skin scrapings into mice is followed by an outcome which is negative or nearly so, save in mice already bearing spontaneous neoplasms; but the following discussion of those tumors (whether they be local metastases or independent growths) which arise in the mammary glands of mice already bearing one spontaneous neoplasm, shows that no particular significance can be attached to the development of tumors in these experiments.

CONTROL

Concerning the numerical relation of mice bearing multiple spontaneous tumors to those bearing but a single growth, Dr. Wm. H. Woglom of the George Crocker Special Research Fund at Columbia University has furnished us data from 800 tumor mice. Of this number, 528 had one tumor each, 193 had two, 68 had three, 8 had four, 2 had five, and one bore six tumors. Thirty-four per cent of the whole group, therefore, had multiple tumors. In a similar smaller series from the Gratwick Laboratory approximately the same condition obtained. Of 137 spontaneous tumor mice 33 per cent were multiple: 92 bore one tumor each, 23 had two tumors, 11 had three, 8 had four while there were 3 mice with 5, 6 and 7 tumors respectively. These results are considerably higher in their proportion of multiple tumor mice than those reported by Apolant, Murray, and Haaland (12 per cent to 17 per cent).

The time element is not considered in the preceding paragraph. Nine multiple tumor mice chosen at random from Dr. Woglom's series had developed the second tumor during a period between 7 and 140 days after the first was recorded, the average period intervening being 47 days. From our own records we have segregated 24 multiple tumor mice, in which the second tumor appeared after 7 and before 159 days, the average being 49 days. The results from two sources are thus in close correspondence, and afford no grounds for believing that the experimental spontaneous tumor mice reacted differently from the other mice.

The spontaneous tumor mouse, though obviously the best experimental material from the standpoint of susceptibility, is less suited for experiments whose results may be spread over a period of several months. Thus, 50 tumor mice not the subjects of experiment died between the first and seventh month after the first tumor was recognized, and the average period of survival was only 2.16 months.

SUBCUTANEOUS IMPLANTS OF LIVING MITES

Instead of implanting skin scrapings containing hair with living and dead mites, living mites were now picked individually from the hairs and implanted directly. Scrapings were obtained as before, and the mites were caught on the point of a very fine dissecting needle with the aid of a binocular dissecting microscope and immediately transferred, one at a time, to the mammary tissues of the mouse through a slit in the skin. Only an occasional hair was introduced with the parasites.

Eight spontaneous tumor mice were thus implanted subcutaneously in the mammary region, at a point distant from the tumor, with an average of 16 living mites per mouse. In five cases, the mites were obtained from the same mouse into which they were implanted, in three cases from other mice. One of the tumor mice died after 5 days with a small additional tumor close to the implanted area. Another survived 7 weeks and had at autopsy a minute adenoma where the implant was made. All the other mice, of which one survived only 10 days, the rest from 1 to 6 months, were negative.

Nineteen old normal females, exact age and condition unknown, were implanted in various subcutaneous locations in the mammary region with living mites, the number averaging

21 per mouse. The average survival was a little over one month and the result was entirely negative.

Eleven mice, similar to the preceding, received an average of 40 mites per mouse, chiefly in the left axillary and left inguinal regions, together with a small quantity of kieselguhr. A crushing pressure was then applied to the subcutaneous tissues of the region by means of heavy iron pliers. The average survival was 100 days. Three of the mice developed tumors, within 14, 18, and 63 days respectively.

Forty living mites were picked from the skin scrapings of old mice, and placed upon a small bit of breast tissue from an old mouse; the whole was ground thoroughly in an agate mortar with a very small quantity of kieselguhr, and the resulting small, dry, waxy mass was divided and implanted beneath the skin of the axilla in 7 spontaneous tumor mice. The average survival was 46 days and the result entirely negative.

INJECTION OF MITES GROUND IN SALT SOLUTION

Seven spontaneous tumor mice, some with multiple tumors, were injected in the vicinity of the nipple with material made by grinding living mites with salt solution in a mortar, from 30 to 50 mites being used for each mouse. A fine needle was used in the endeavor to inject the mammary ducts directly, but the procedure was so difficult that the larger portion of the dose was as a matter of fact, deposited subcutaneously. The mice survived for from 11 to 155 days, the average period being 70 days. Three of the 7 mice developed each an additional tumor; two of these became recognizable 15 days after injection, and one of them was in the field of the injection. The other mouse developed an additional tumor after 4 months, at a distance from the injection.

CONTROL

As a control to the preceding group, 8 spontaneous tumor mice were injected with salt solution through the nipples. Again the solution became in part subcutaneous, and in neither case was it possible to be certain when the dose had reached the interior of the mammary gland, and when it had been deposited subcutaneously. In this control, from one to five mammillae were injected; the mice survived from 3 to 69 days, the average period being only 38 days. Two of the 8 mice developed one additional tumor each; one of these arose within 13 days in close proximity to the injected nipple, the other within 36 days and far from the points of injection.

ATTEMPTED WATER INJECTIONS THROUGH NIPPLES

It is of course much more difficult to force water through the nipple duct by means of a glass capillary pipette, than it is to introduce air. Ten old breeders were injected with small quantities of tap water, but it was doubtful whether the fluid reached the interior of the gland. The result was negative. After some improvement in the technic, 11 old females past the breeding age were injected, without any certain control over the location of the injected water, which certainly lay in part subcutaneously. The average survival period was 114 days, and 6 tumors developed among 4 mice.

SUBCUTANEOUS INJECTIONS OF TAP WATER

These led to the early death of the mice, probably from infection, 6 out of 11 mice dying within 10 days; the rest survived several weeks, and were negative. As compared with air, only small quantities of water can be held subcutaneously unless special precautions are taken to prevent its return through the needle wound. About 1 cc. was injected, part of which soon escaped.

MAMMARY INJURY AND TAP WATER INJECTIONS

The inguinal and adjacent breast tissues were cut in many places with a very small flat perforator, by punctures through the skin, and tap water was then injected through the smallest hypodermic needle directly into the gland; considerable bleeding occurred. Ten virgin females about 10 months old were thus treated, but no tumors have developed during 7 months, in the 8 surviving mice.

In the preceding trial, though the instrument used had the smallest blade obtainable, it did not appear to cause the minute and multiplied injury to the alveoli which was desired. Another method was therefore tried, a glass capillary pipette being used, with a broken point as fine (60–70 microns) as was consistent with the delivery of a stream of water from a glass syringe on strong pressure of the piston. Nineteen mice about 11 months old, most of them with lactating glands and in their first and only parturition, were pierced many times in the mammary region of the left side with the pipette while it was delivering a steady stream of clean tap water. The broken edges of the pipette were counted on to cut the secreting epithelium while it was irrigated with water. No measure of the injury thus achieved was attempted. The mice were treated at different dates, as their glands came into proper condition; 5 to 6 months have elapsed, 16 survive, and the result to date is entirely negative.

FEEDING WITH SKIN SCRAPINGS CONTAINING LIVING AND DEAD MITES

Nine normal female mice, from 8 to 14 months of age, abandoned as breeders, were fed on six occasions during 21 days with the scrapings from the ventral skin of 3 tumor mice and 27 normal mice of various ages, though chiefly old. The scrapings contained, as usual, the short hair left after clipping and great numbers of mites, both living and dead. All the mice died within 44 days, and only one showed definite mammary reaction, in the form of a few small adenomata. One had a small lung adenoma.

PRESSURE INJURY TO THE MAMMARY GLAND

Folds of the skin including portions of mammary tissue were seized with hemostats and heavy pressure applied in 20 old females past the breeding age. The result was negative.

INJURY AFTER FEEDING WITH MOUSE SKIN

Sixteen old normal females, no longer breeding, were fed with the skin of other old mice. The feedings covered a period of 29 days, during which skin from 60 mice, prepared as in previous experiments was fed on fourteen occasions, and nearly all the skin offered was completely consumed at each feeding. It contained varying numbers of mites, in some cases very few, in others many; the total amount of living and dead mites consumed, was, however, large. Lice also were occasionally present. On the day of the eighth feeding, the mammary region about each inguinal region was punctured six to ten times with a file, as described in earlier experiments. Two days later, on the day of the tenth feeding, the left axillae were treated in the same way. Finally, on the day after the last feeding of skin, and 30 days after the beginning of the experiment, the axillary and inguinal tissues of the 10 surviving mice were subjected to heavy pressure by means of pliers.

After $8\frac{1}{2}$ months, 6 of the mice still surviving, no tumors have developed.

At this stage of the experiments, it appeared that despite negative or equivocal results a slight increase of incidence had been obtained, irregularly distributed among the treated mice, and that this effect could more reasonably be attributed to agencies incidental to the introduction of parasites than to any specific action of the latter themselves. It seemed, in fact, an expression of the inconstant and occasional effect of trauma. The necessary injury involved, or even the mere opening of the skin and consequent exposure of the subcutaneous tissues to the air (in one case water with its dissolved air), or both combined, were at least as plainly indicated as probable active agents as were the parasites. In fact, while it is difficult to establish such a negative, it seems fairly certain that the acarines of the skin of mice play no essential part in causing mammary tumors; for although introduced by feeding and by implantation, living and dead, in small and in large numbers, intact and ground up, combined with definite tissue injury as well as with a minimum of injury, the few tumor reactions which follow are no more closely linked with the acarines than with injury or exposure of the tissues to air.

Accordingly, a series of experiments was devised to make more prominent the latter factors, in which it was proposed to inject ordinary air into the ducts and alveoli of the mammary gland through the nipple. By the use of a fine metal hypodermic needle, and later of glass pipettes drawn to capillary size (20–50 microns diameter) and with the aid of lenses, the gland could in some cases be slightly inflated with air. The attempt involves fatalities from air embolism sometimes reaching 60 per cent; by autopsy on such mice, air may occasionally be plainly seen within the gland, but more often honeycombing the subcutaneous connective tissues outside it, for the duct is so small that the pipette can not usually be inserted accurately within its lumen without laceration of the nipple, and chance determines to a large extent the distribution of the injected air, a very slight malposition of the pipette sending the air subcutaneously.

ATTEMPTED AIR INJECTIONS INTO THE MAMMARY ALVEOLI

In May, 1916, five normal breeding female mice, one year of age, were injected through the third and fifth pairs of nipples, and by means of a glass hypodermic syringe and metal needle, with small quantities of air, some of which entered the mamma and some the subcutaneous tissues. The mice continued to breed more or less until death.

One died after 81 days and was eaten by its cage mates; it had had no tumor at the last examination, about one month before death. The other four all developed mammary growths between the third and sixth months after injection, bearing a total of 12 tumors, of which 7 arose or were found between the third and fourth month; at least 8 of these 12 neoplasms were in the immediate vicinity of the point of injection. The tumor mice died at ages between 16 and 19 months; average age 17.4 months.

In May, 1916, 6 breeding mice 12 months old, which had recently been nursing young, were injected with air as in the preceding experiment, save that a finely drawn glass pipette and a rubber pressure bulb were used. The injection appeared to be successful in lodging part of the air within the ducts as well as subcutaneously, in each mouse. Of 10 mice thus treated, 4 were killed by embolism, and autopsy showed air within the mammary gland of all. The 6 survivors were held in one cage without males after the injection; 7 tumors arose in 5 of these mice between the third week and the sixth month of the experiment, though only 4 of these 7 tumors (in 4 mice) were in the injected region. The tumor mice died at ages between 14 and 22 months; average age 18 months.

Thus 9 of 11 mice developed 19 tumors during the 6 months following injection. Twelve of these 19 tumors, in 8 of the 9 mice, were located either at a site of injection or in the mammary region to which an injected nipple belonged.

CONTROLS

These mice subjected to air injection through the nipples were not directly controlled; the two experiments were merely tentative, in a groping endeavor to find an effective method. Not much success was anticipated, and the result after some 3 months was supposed to be negative, but the mice were nevertheless held. The incidence finally reaching over 80 per cent, however, a control is required, although such an incidence is rare even among inbred cancer strains of high rate. Miscellaneous breeders have usually been abandoned before reaching the necessary age, the few figures available indicating that 10.6 per cent of the breeding stock in question produce tumors before 14 months of age. Comparable miscellaneous virgin mice show, up to 14 months of age, an incidence of about 8 per cent, based on over 140 mice in about a dozen groups or lots. One hundred nine of these mice, during the age period between 16 and 18 months, raised the incidence to 21.1 per cent. But since tumor strains are irregularly distributed among them, it becomes necessary to seek further evidence. The groups showing the highest incidence may be selected; three such lots comprising

$6 + 5 + 3 = 14$ mice having developed $2 + 2 + 1 = 5$ tumors, or 35 per cent, at an age of 17 months, this is the maximum incidence that may be expected among accidentally selected small lots of non-breeders. But the experimental mice were breeders, and as a rule breeding undoubtedly increases tumor incidence.[2] The figures cited above indicate only a slight increase for the stock from which the experimental mice were taken. Even allowing more liberally it is scarcely possible to avoid the conclusion that an external factor has been introduced and has reinforced the inherent predisposition among the experimental mice to develop tumors. A random choosing from this laboratory stock of 11 mice, 9 of which were destined to develop spontaneous tumors before 17 months of age, is not impossible but is a contingency extremely remote.

As for multiple tumors in mice not the subject of experiment, records of 141 spontaneous tumor mice show an average of 1.57 tumors per mouse. The experimental mice have 2.1 per mouse. Seven of the 9 tumor mice were examined for metastases, which were found in 3, or 42.8 per cent. Among 112 spontaneous tumor mice metastases occurred in 41 per cent.

Virgin mice and young breeders do not react to the air injections, and older adult mice injected after their one and only parturition perhaps occupy an intermediate position, as is indicated by the following:

Fourteen miscellaneous virgin mice 7 months after the injections, having reached an age of 20 months, have produced but one tumor, 12 mice surviving. Furthermore, 21 virgin mice of an inbred strain of low tumor rate, injected at 3 months of age, have produced no tumors during the succeeding 6 months, all surviving. Seven females of a very high tumor strain were injected after the first parturition, at age 3½ months; they then bred continuously to an age of 11 months, except one which died at 9 months without a tumor. All the others save one developed tumors, but neither the tumor incidence nor the age period in which the tumors first became manifest in other mice of the strain was measurably altered. It appears that injections into young mice does not influence them even when they arrive at cancer age.

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[2] Lathrop and Loeb: This Journal, vol. i, no. 1, page 1; shows strains varying in this increase up to 35 per cent.

Ten females one year old were injected after their first parturition, subsequent to which they were held without further breeding. Four to five months have elapsed, and four mice have developed tumors, seven still surviving. At present this result is not unequivocally beyond the incidence which may possibly be expected in controls. Since the mice were of mixed ancestry, direct controls by corresponding lots comprising small numbers of mice are inconclusive. Only by an overwhelming incidence in the experimental animals may a definite positive result be inferred, and therefore the earlier results on intensively breeding mice are not yet extended to primiparous breeders.

AIR INJECTED SUBCUTANEOUSLY

This procedure was intended to check the trials wherein air was injected by way of the nipple with a capillary pointed glass pipette. It aimed to test the effect of the almost continuous presence of free air beneath the skin but not inside the mammary gland, during several months, without injury to the nipple.

Some 70 virgin mice, most of them of cancer age, have been injected repeatedly beneath the skin of the breast regions and elsewhere with ordinary air, by means of a glass hypodermic syringe fitted with the finest metal needle. The procedure was repeated at intervals, so that many of them have received 8 to 10 cc. in 16 or more injections during some 8 months, and were seldom without some free subcutaneous air during this time. The air disappears slowly, presumably by absorption chiefly and remnants of an injection of several cubic centimeters may still be recognizable after 2 weeks. One lot was allowed to breed after the second month. In another lot injury by heavy pressure on the mammary tissues preceded 3 of the 9 injections made. Though several tumors have occurred and many of the mice still survive after 8 months, the results at present are to be regarded as negative.

MAMMARY INJURY IN PRESENCE OF AIR

The left axillary skin of 20 virgin females 11 months old was slit and the axillary fat and other tissues were opened by snipping several times with scissors; these tissues were then pricked many times with a capillary glass pipette through which a strong current of air was continually passing. Four mice have developed tumors at the fifth, sixth, seventh and ninth months, respectively. Nine mice survive after nine months.

The same procedure was carried out with 20 old females which had finished breeding, save that the punctures were made through the unbroken skin and with a much finer pipette. The mice all died during the succeeding 3 months and were negative.

Twenty virgin mice 9 months old were treated in a similar way, except that the pipette was of much larger size than in the preceding trials, and that the mice were injected in the inguinal region. Injuries to the blood-vessels resulted in the death of 7 of 27 mice from air embolism. Six mammary tumors have developed among 4 mice, arising between 2 weeks and 5 months after the beginning of the experiment; another developed, within a few days, several lymphomata among the cervical and mediastinal tissues, and all the axillary and inguinal lymph nodes were enlarged. Nine months have elapsed and 9 mice still survive.

This experiment was controlled directly by 20 mice of the same lot from which the treated mice were taken. For some reason, however, they have succumbed more rapidly than the latter; all have died, the average survival period being but 86 days. No tumors developed among them.

INJURY TO NIPPLES

Glass pipettes were drawn to needle points without obliterating the lumen, and the tips were broken off; these pipettes, which would pass into the nipple duct, though not without a scraping injury, were used as needles to pierce the nipple through its fleshy part as well as longitudinally along its duct; nothing, however, was injected. The duct was never patent, and in nonbreeding mice could usually not be recognized with a x-3 lens.

Three or four of the nipples on the left side in 18 virgin mice 10 months old were each pierced several times with such pipettes, intersecting the duct here and there. After 5 months, only 3 survive, and no tumors have resulted.

Ten females 13 months old, soon after their first and only parturition, were treated in the same way, save that nearly all the nipples in each mouse were pricked; half these mice had lost their young, while the others were nursing their litters. Six mice survive after 5 months, during which 3 have developed tumors between the sixth week and the third month, in the region of the pierced nipples. One of the mice had 3 or 4 tumors.

This procedure was intended to show the effect of the capillary glass point without a current of air, used exactly as was a similar point carrying air. While the number of mice is few, the trend of the result is probably correct, in indicating little or no effectiveness for the simple trauma without air. The controls already discussed apply here. Possibly after further breeding the same procedure would raise the incidence considerably.

SUBCUTANEOUS DEPOSITS OF CHARCOAL

Nineteen breeding female mice of an inbred tumor strain of low rate received, at 7 months of age, a quantity of powdered wood charcoal in the left axilla. The mammary glands were in active function. The left axillary tissues were then subjected to pressure by means of pliers, crushing the charcoal into the tissues. The mice continued to breed. Three months have elapsed and one tumor has developed, not however in the axillary region. Other experiments with charcoal are in progress.

DISCUSSION AND SUMMARY

More than 1100 mice have been used in over 60 experiments in irritation and trauma, and most of them are dealt with in this paper, which is in the main a record of negative results. The experimental methods were not chosen at random, but were in the beginning implied by the observation that the common acarine ectoparasites of mice were to be found dead beneath the skin in old animals. The attempts to demonstrate these parasites as agencies capable directly or indirectly of starting epithelial tumors in mice resulted in a failure which coupled with the extent and thoroughness of the experiments, is complete enough to be convincing to the writer with respect to the parasites in question. Nevertheless, in some of the various experiments centering about these parasites, notably file punctures, which do not necessarily involve the parasites, tumors developed in sufficient numbers to indicate a moderate increase of incidence due to the artificial procedures followed, and plausibly and naturally connoting the general effect of trauma or irritation. The trauma in any degree effective in these cases can not be very specifically defined, and the procedure, when carried out with apparent uniformity, has a negative more often than a positive result, as far as the number of individuals is concerned; the effective specific injury occurring occasionally, however, with a given procedure and in a suitable subject, a tumor eventually arises therefrom. Though some foreign bodies capable of causing chronic irritation were introduced, the evidence points rather to the acute discontinuous trauma.

Since the experimental trauma always admitted air to the injured tissues, an attempt was made to combine and intensify the two factors, air and trauma, making the injury more definite and specific. By the injection of air through the nipple duct into the mammary units, it was thought that perhaps a highly intensified or specialized trauma might be caused by stretching the alveoli in intimate association with air. Such injections could be obtained in a considerable percentage of cases with glass pipettes drawn to microscopic points accompanied usually or always with air, subcutaneous but extra-mammary in position. Two protocols comprising 11 females of indefinite ancestry with an intensive breeding record, injected in this way at one year of age show 9 mice (81 per cent) bearing 19 tumors developed at widely separated dates during the succeeding 6 months. Sixty-three per cent of these tumors in 8 of the 9 mice, were collocated with mammary regions to which one or more injected nipples belonged, while the rest arose in distant regions.

Controls of breeding mice of equal age are lacking. Breeders up to 14 months show an incidence of 10.6 per cent. The tumor ancestry contained in these mice may be measured at a maximum by selecting similar lots of non-breeders showing the highest incidence; three such lots, comprising 14 virgin mice, show at an age of 18 months an incidence of 35 per cent, to which should be added a presumably small factor for the influence of breeding.

Virgin mice, however, have not reacted at all to the same treatment, and a repetition upon primiparous mice has not yet yielded equivalent results. That breeding mice should be more susceptible is to be expected, and the negative result with virgins and the high incidence with intensive breeders may be attributable to the breeding factor alone. Nothing definite supports the speculations which might be made concerning the influence of season, temperature, or any accidental, slight, and unrecognized variations in the technic. Possibly the method may succeed or fail erratically from causes not yet apparent.

Mere subcutaneous injections of air into the mammary region or elsewhere in the mouse, even when continued for months, are without effect in producing tumors. Mere punctures of the nipple with a finely pointed glass pipette, or the passing of the point into the nipple duct, without a current of air passing, are probably without marked effect. But when a current of air issues from such a pipette and the subjects are well into cancer age and have repeatedly produced young, a high tumor incidence occurs in the next few months, perhaps whether the air does or does not find its way into the mammary gland. Macroscopic quantities of air suddenly driven into the mammary alveoli would doubtless cause an extensive and well distributed trauma; the point of the injection pipette must, on the other hand, cause a restricted injury, different in kind, presumably very slight and subtle, involving possibly few groups of cells, and in the presence of a blast of air. Whether both forms of trauma are of importance does not yet appear. Since the latter form alone was known with certainty to have been inflicted on every one of the 11 mice, the former is probably subordinate. Whether any of the tumors arising in mammary regions distant from the locus of the injection may share the presumptive relation to the latter which attaches to those tumors regionally associated with the injected nipple is perhaps a debatable question, which can not yet be settled. At present it merely seems highly probable that some form of excitant effective in a material degree is delivered by the air-injury technic described.

These experiments as a whole might be summed up as tending strongly to exclude acarine parasites of the mouse from any concern with the origin of mouse tumors; as showing how extensive a series of acute injuries fails to inaugurate tumors; and in indicating a simple and definite form of trauma which seems to have a material effect in raising tumor incidence in old breeders. Incidentally one may further infer that the effective factor hidden in any mechanical procedure which appears to start tumors is a very subtle one, and that its isolation or identification is a very difficult task.

TUMOR IMMUNITY IN THE CHICK EMBRYO

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From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director

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In a recent communication, experiments were reported in which it was found that adult chicken spleen had no influence upon the growth of mouse and rat tumors in the chick embryo. The tumor and spleen were inoculated simultaneously and allowed to grow for ten days, and the volume of spleen introduced (0.005 gram) exceeded the quantity of tumor (0.003 gram). The amount of spleen was determined arbitrarily, by the dose of mouse spleen necessary to induce immunity in the mouse and also by the comparative size of the seven-day chick embryo and the adult mouse; it appeared that the quantity of spleen used would be more than enough to induce immunity in the chick embryo if this were possible. It was found, however, that although over one thousand fertile eggs were inoculated, with six tumors of varying characteristics, the grafts proliferated without any suggestion of inhibition. The presence of the splenic graft on the allantois induced a striking increase in the round-cell reaction about the tumors; on careful study, however, it was found that the majority of these cells were of the myeloid or granular leucocyte series, and not lymphocytes.

It was pointed out in the paper, in discussing these findings, that, although this reaction had been induced and yet had not influenced the growth of the tumor, it would be necessary to determine whether or not even larger doses of spleen would prevent the tumor from growing. The observations on the proliferative power of tumors growing in the presence of adult chicken spleen had been based upon a study of the grafts in serial section, tumors in the sections containing spleen being adjudged as living when mitotic figures were found.

But in the discussion of an abstract of the paper just referred to, which was presented before the American Association for Cancer Research at its annual meeting in April, 1917, Gaylord pointed out that a tumor showing mitotic figures is not always alive, and that for this reason one would be unable to say, with absolute certainty, whether these tumors growing in the presence of spleen were or were not viable.

Although the presence of numerous mitotic figures in a uniformly healthy tumor showing no necrosis is the usually accepted criterion of its viability and malignancy, Gaylord's criticism is not entirely unjustified in this instance; for while the slides presented at the meeting showed that the tumors fulfilled all the requirements necessary to establish their malignancy microscopically, it was impossible at the time to present further evidence in corroboration.

The following experiments were therefore carried out, to determine:

1. Whether or not adult chicken spleen in amounts greater than 0.005 gram will influence the growth of a tumor when both are inoculated simultaneously into the chick embryo.

2. Whether a rat tumor, after growing in the presence of spleen in the chick embryo for ten days, and showing mitoses, is alive, as demonstrated by its capacity for continued proliferation in the rat.

EXPERIMENTS

The conditions of experimentation were similar to those previously reported; the eggs were derived from the same source, and the technique of inoculation was similar to that employed previously. Inoculations were made on the seventh day of incubation and the grafts removed on the seventeenth or eighteenth day of incubation.

1. The spleen was removed from healthy adult fowls, minced with scissors, drawn up into a finely graduated syringe, and inoculated in amounts of 0.02 cc. upon the allantois of seventy-

nine embryos, together with 0.003 gram of a healthy Jensen rat sarcoma, the same tumor used by Dr. J. B. Murphy in his experiments. Twenty-two control eggs were injected with a similar amount of tumor alone. Ten days later the eggs were opened, and the grafts removed; of the controls, fourteen were living, and of these eight bore tumor grafts.

The tumor, in serial sections where both tumor and spleen were present, was in a healthy growing condition and contained many mitotic figures. The spleen grafts (fig. 1), as a rule, showed large healthy areas, but there were also large necrotic areas present, as is generally the case when very large quantities of tissue of any type is inoculated. No difference in the reaction on the part of the wandering cells (fig. 2), which were abundant, or in the condition of the tumor, was noted on comparison with those obtained previously with the smaller dosage of spleen. On comparing these grafts with the controls, no difference in their condition could be discovered.

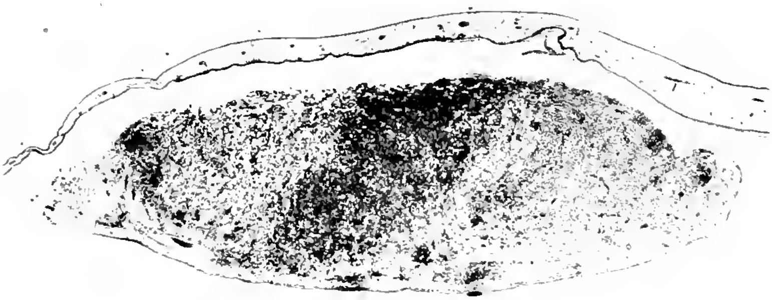

FIG. 1. SPLENIC GRAFT ON THE ALLANTOIS

Arising from the inoculation of 0.02 cc. of splenic mush. A small portion of Jensen rat sarcoma is seen at one end of the graft. × 10.

2. A series similar to the above was inoculated, using 0.003 gram of a Jensen rat sarcoma and 0.02 cc. of a preparation of adult chicken spleen in each egg. A control series of eggs was inoculated with the tumor alone. When the grafts were re-

moved ten days later, seven of the largest of those growing with spleen were taken out aseptically from the egg, and each was cut into two parts, one of which was inoculated into twelve rats, the other being saved for microscopic examination. One of the grafts resulting from the control series of eggs was treated in a similar way. The boxes and the portion of the graft saved for section were given identical numbers.

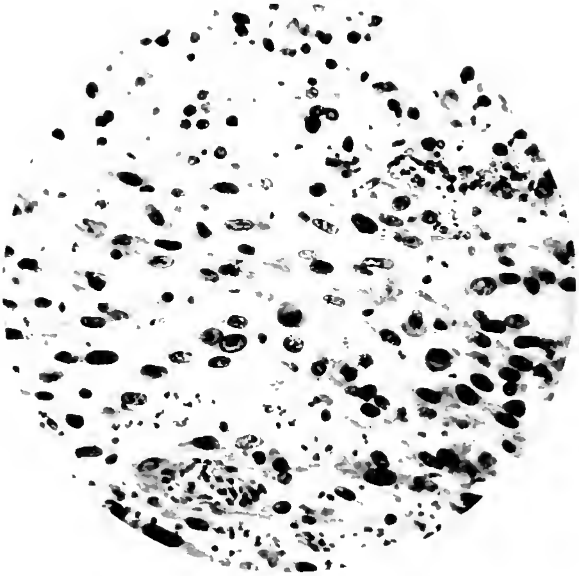

FIG. 2. JENSEN RAT SARCOMA FROM 1

Showing the wandering cell reaction and numerous mitotic figures in the tumor cells. × 300.

Tumors (fig. 3) developed in three of the series of rats and in the control; these were removed, their identity proved by sections, and one of them was inoculated into a second series of rats, where normal growth resulted. The chart (fig. 4) shows the growth of the tumors in the rat, after growing with spleen and without spleen in the chick; there is no difference between the two series.

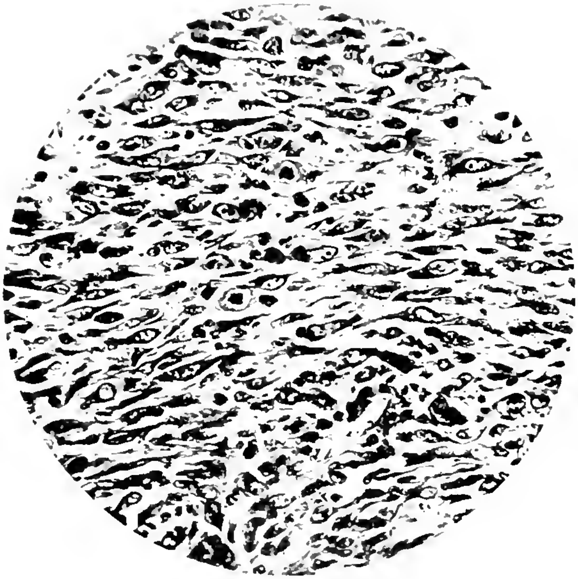

Fig. 3. Jensen Rat Sarcoma in the Rat

Resulting from the inoculation of a portion of the graft shown in 1 and 2. × 300.

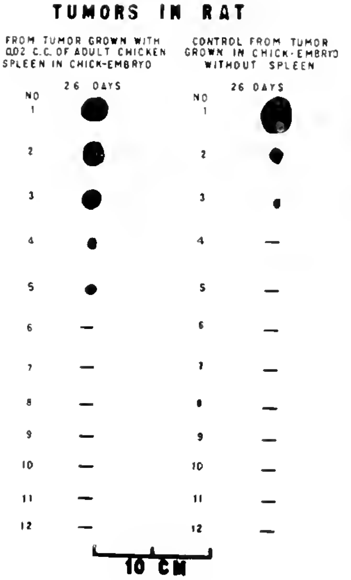

Fig. 4

Examination of sections of those grafts which had grown in the chick embryo in the presence of spleen, and from which the inoculations were made, was of interest. Some of these contained merely the tumor in a healthy condition, while others contained both tumor and spleen. The grafts from which the three series of rats that bore tumors were inoculated, showed in the sections large healthy grafts of spleen.

Another experiment similar to this was carried out, using adult chicken spleen in the amount of 0.01 cc. and the Jensen rat sarcoma. In this series, also, the inoculations into rats of grafts that had grown with spleen in the chick, developed a certain number of tumors. The sections of the grafts, from which these tumors resulted in the animal, contained splenic grafts in a healthy viable condition.

SUMMARY

There appears to be little necessity for a discussion of the results of the experiments presented above. The total number of fertile eggs employed was about three hundred. The results were uniform, and prove that the tumors exhibited no signs of inhibition when they were grown on the allantois of the chick embryo for ten days, in the presence of adult chicken spleen in amounts up to 0.02 cc. This was demonstrated not only by the microscopical appearance of the tumors, but by their unmodified growth when returned to the animal to which they are native.

THE RELATION OF INDUCED CANCER IMMUNITY TO TISSUE GROWTH AND TISSUE DEGENERATION

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From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director

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Although certain investigators have asserted that they produced immunity against transplantable animal tumors through the agency of autolyzed cells (1) and heterologous tissues (2), it is the consensus of opinion that immunity occurs only when the immunizing material consists of live cells derived from the animal species in which the tumor originated. Assuming that living cells are prerequisite for the production of immunity, then immunity may be due either (a) to the growth or growth metabolites of the injected material as suggested by Haaland, or (b) to the death and degenerative metabolites of the injected material, or (c) to growth followed by death with their respective metabolites. Other factors such as the lymphocytes, stroma reaction, etc., are not considered in this paper, which is concerned only with the question of life or death of the tissues employed.

Dead cells are not capable of inducing the resistant state, a fact which lends force to the supposition that immunity may be due to growth or the metabolites of growth. On the other hand, tumor cells, which show a rapid and prolonged power of growth, do not invariably have the power of producing immunity. If growth or the metabolites of growth are the prime factors in the production of immunity, then it would be natural to suppose that of two normal tissues, the one showing, after injection, the longer duration of growth and the greater increase in bulk would give the higher percentage of immunity.

In the first experiment, the immunizing power of cartilage and bone was compared with that of fetal skin. Of these two tissues, the bulk of the injected material being equal, cartilage and bone persist longer in the tissues of the injected animal, and reach a larger size, than does fetal skin. Zahn, Leopold, Loeb, and others have observed that the growth power of embryo cartilage is more pronounced than that of other embryonic tissues. Thirty-six rats were injected in the subcutaneous tissues of the right side with 0.05 cc. of rat embryo skin emulsion. In a second series, 36 rats were injected in the same region with a slightly larger dose (0.06 cc.) of rat embryo cartilage and bone, freed from the soft parts by repeated scraping. Ten days thereafter the animals in each series, together with 36 normal untreated rats, were inoculated by needle in the left axilla with 0.003 gram[1] of the Flexner-Jobling carcinoma.

The results of this experiment (Chart 1) showed that immunity was present in 39 per cent of the animals injected with embryo skin, as compared with 19 per cent in the group injected with fetal cartilage and bone, and 8 per cent in the nontreated controls.

The experiment indicates that growth of the bone and cartilage was not sufficient in itself to produce a degree of immunity equal to that produced by embryo skin. It suggests that possibly a greater activity in the production of metabolites exists in the embryo skin owing to the fact that, bulk for bulk, more cells are present and in active proliferation in this tissue than in bone and cartilage. It may be maintained that connective tissues, such as bone and cartilage, do not produce immunity, though both the Buffalo and Jensen rat sarcomata, pure connective tissue growths consisting mainly of rapidly dividing cells, have the power of inducing the resistant state in high degree.

Large doses (0.05 cc.) of embryo skin injected for purposes of immunization are subjected to early degenerative changes resulting in the formation of large amounts of necrotic material.

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[1] In previous publications from the Imperial Cancer Research Fund and from this laboratory, the inoculation dose, when the needle method is used, has been estimated as 0.01 or 0.02 gram; but such grafts have recently been found, as a matter of fact, to weigh about 0.002 and 0.003 gram respectively.

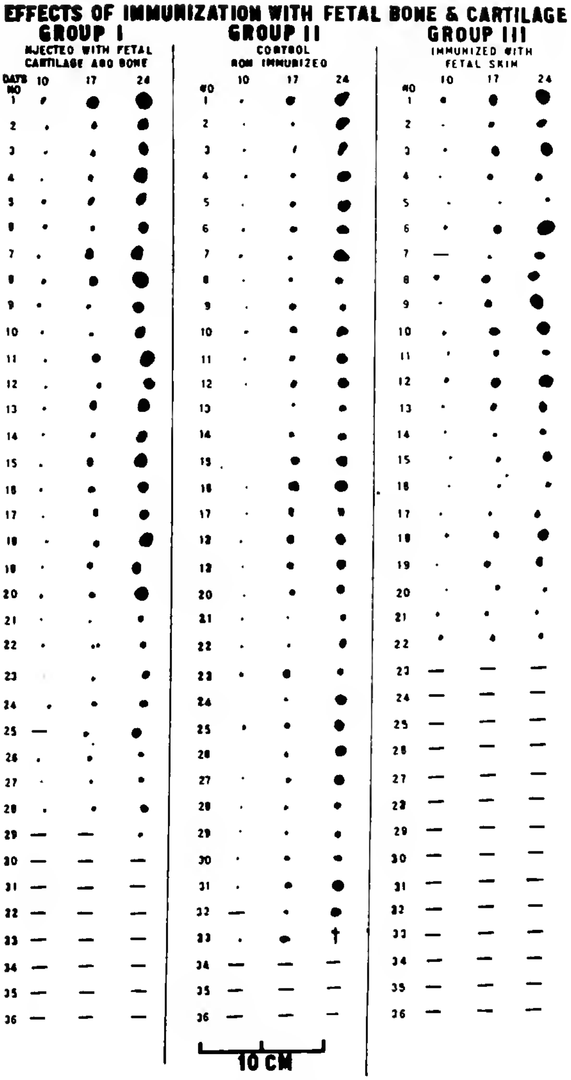

Chart 1. Showing Difference in Degree of Immunizing Power of Similar Doses of Fetal Cartilage and Bone as Compared with Fetal Skin

This is shown in the result of a study of the immunizing material and the tissue reaction about it, undertaken partly to ascertain whether immune animals differed from susceptible anima's in their reaction to the immunizing agent.

An emulsion of rat embryo skin was injected into the subcutaneous tissues of 36 normal rats in doses of 0.05 cc. On the following day, and on each succeeding day for eight days, the injected material and the overlying skin were removed *en bloc* from 4 of the animals by operation, and the tissue studied microscopically in serial section. Ten days after the introduction of the embryo skin emulsion, the animals were inoculated with the Flexner-Jobling carcinoma. In a second series of rats injected with rat embryo skin emulsion and inoculated with the Flexner-Jobling tumor ten days later, the injected material was removed from 12 animals each on the tenth, fourteenth, and twentieth day after its introduction and submitted to microscopical examination.

Roughly estimated, fully two-thirds of the injected material showed complete degeneration before the fifth day. The surviving epithelium did not retain its healthy condition much over nine days, and only one healthy epithelial cyst was noted after ten days. There was practically complete degeneration of the injected material in fourteen days, and in twenty days after the immunization organization was far advanced. This is in sharp contrast to the results of injecting small bits of embryo skin which grow and retain their viability for weeks. Aside from individual variations, there was no difference noted in the tissue reactions, or in the viability of the injected tissues, in rats which proved to be immune as compared with non-immune rats. The best preserved tissue was noted in an animal with a growing tumor. This shows that it is impossible to determine by the local reaction of the animal toward the immunizing agent, or by the condition of that agent, whether an animal will be susceptible or immune to a transplantable tumor. Woglom, in an unpublished investigation, had previously come to the same conclusion.

Accepting temporarily the premise that immunity is due to the absorption by the animal of some products of cell degeneration, we made an attempt to produce immunity by causing an increased amount of absorption of the products of tumor necrosis. To insure the production of large amounts of degenerative tumor products, massive doses of tumor material were used for inoculation. In these experiments the Flexner-Jobling rat carcinoma, a tumor which does not produce concomitant immunity, was employed. All secondary inoculations were made into the subcutaneous tissues by needle.

The truth of the working hypothesis could be determined only by using a tumor which, like the Flexner-Jobling carcinoma, does not produce concomitant immunity under the usual conditions of growth. As is well known, Bashford has divided transplantable tumors into two classes, one of which grows for a period and then recedes, while the other grows progressively. The first class often produces concomitant immunity, the second class usually does not. Certain tumors, therefore, are able to produce the resistant state, and from certain investigations under way in this laboratory, but still incomplete, it is not improbable that the immunizing power of tumors may fluctuate.

In the first experiment, a series of 67 rats was inoculated subcutaneously with 0.003 gram of tumor, and after eighteen days' growth the tumors were extirpated from 21 of these rats and replaced in the shoulder region of the same animals, in order to produce a large amount of degenerative tumor material. From a second group of 24 rats the tumors were extirpated and not replaced, while in a third group of 22 animals the tumors were not interfered with. Eleven days later the animals of these three groups and 24 normal control rats were inoculated with 0.003 gram of the same tumor strain. The results as depicted in Chart 2 show that increasing the amount of degenerative tumor material (removal of the tumor and replacement in the axilla) does not materially increase the percentage of immunity, the proportion in this group being 19 per cent as compared with 22 per cent in the group not operated upon. This slight fluctuation is well within the limits of ordinary variation, and is not attributable to the experimental procedure.

In the second experiment, the procedure was modified as follows: One group of 18 rats was injected in the subcutaneous tissues by syringe with 0.5 cc. of tumor emulsion and a second group of 18 control animals was inoculated by needle with 0.003 gram. Ten days later, the animals of each group and a normal control group of 18 rats were inoculated with 0.003 gram of the same tumor strain. As is shown in Chart 3, 6 per cent of the animals receiving the initial large dose, and 17 per cent of those

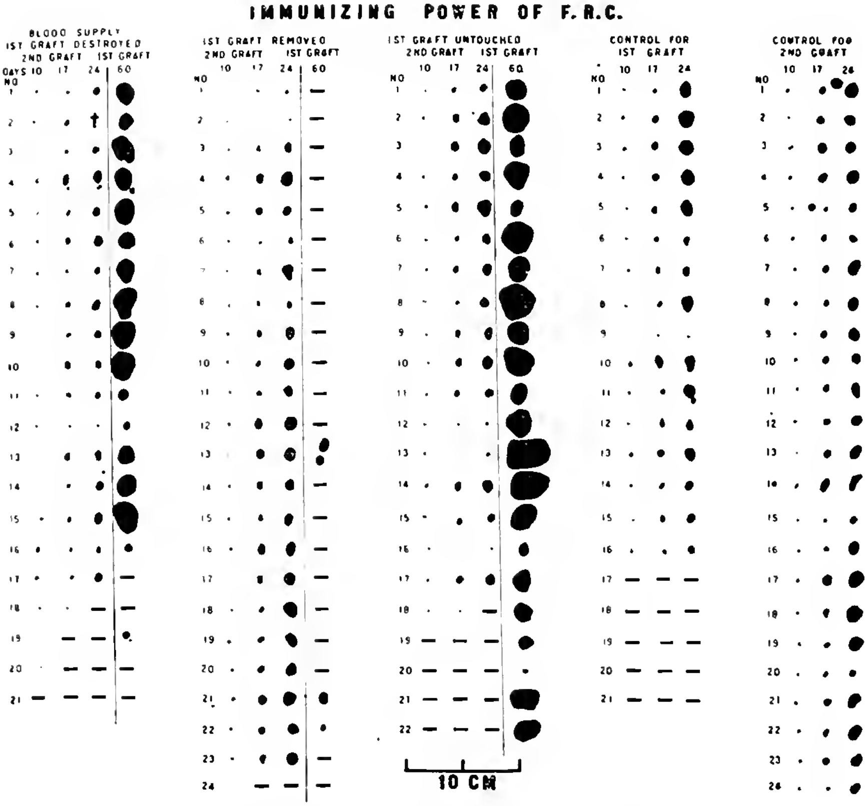

CHART 2. "BLOOD SUPPLY OF FIRST GRAFT DESTROYED" INDICATES THAT THE GRAFT WAS EXTIRPATED AND PLACED IN A NEW POSITION

The sixtieth day of the first graft (fourth row of first three columns) is the same day as the twenty-fourth of the second graft.

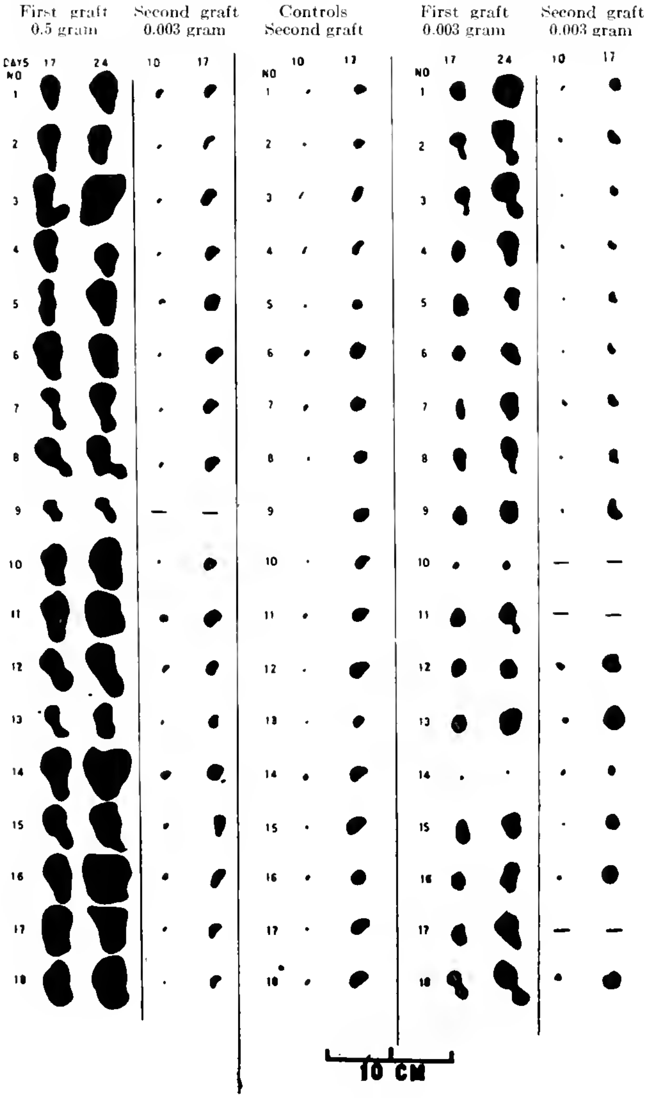

CHART 3. SHOWING ABSENCE OF EFFECT OF INITIAL LARGE AND OF INITIAL SMALL INOCULATION DOSES OF TUMOR UPON THE GROWTH OF SUBSEQUENT GRAFTS

receiving the initial small dose, were immune to the second inoculation. This is opposed to the observation of Bridré (3), who found that an initial large dose caused immunity against

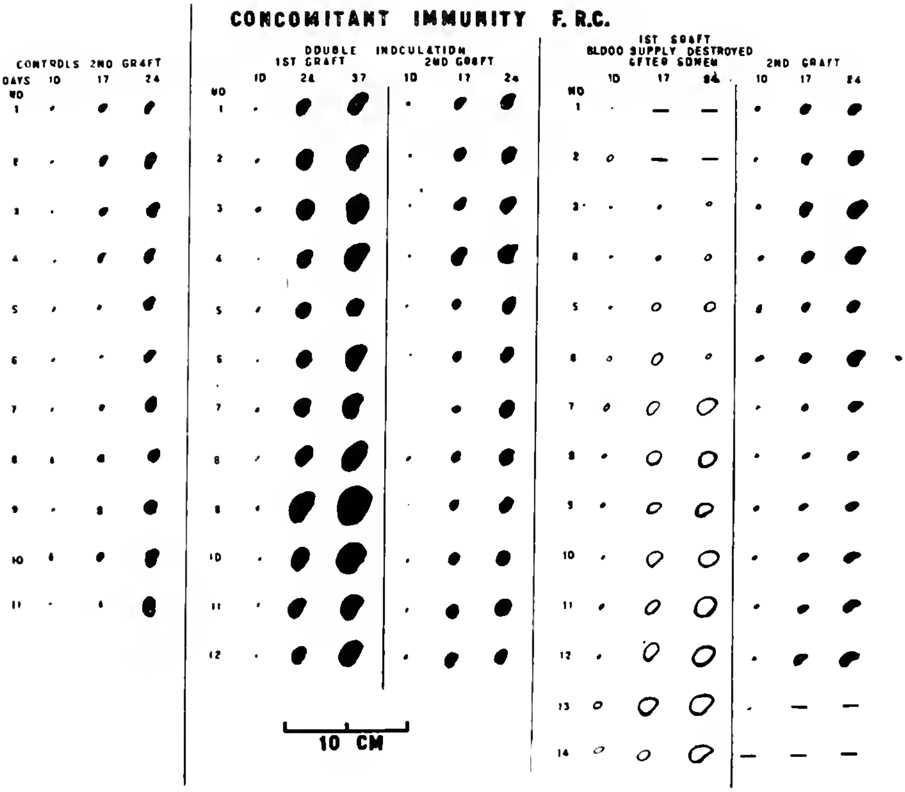

Chart 4. The Size of the Tumor Before Operation Was Equal to Those Shown under Twenty-four Days of First Graft Double Inoculation

The thirty-seventh day of the first graft double inoculation is the same as the tenth day of the second graft of the double inoculation as well as the tenth day in both columns of the blood-supply-destroyed group.

a subsequent graft of the same strain while small doses did not do so. If our working hypothesis were correct, we should have expected to find, as did Bridré, the greater percentage of immunity in the group receiving the larger initial dose of tumor, since large doses of tumor give rise to more degenerative products than do small doses.

Finally, an attempt was made to cause immunity through the production of large amounts of necrotic tumor material by means of obliteration of the blood supply of the tumor after the method of Bowen (4). Twenty-six rats bearing eighteen-day old Flexner-Jobling carcinomata were selected for the experiment. In 14 of the rats, the blood supply of the tumor was destroyed, the remaining 12 animals serving as controls. Eleven days after the operation, these animals and 11 normal rats were inoculated with 0.003 gram of the same tumor strain.

The results of the experiment (Chart 4) indicate that in spite of destruction of the blood supply, enough cells survived to permit further growth of the tumors and, notwithstanding the fact that large amounts of degenerative material were produced and absorbed, very slight (14 per cent) immunity resulted from the procedure; each set of controls showed 100 per cent takes.

CONCLUSIONS

Induced immunity to transplanted cancer is not solely due to growth or the metabolites of growth of the immunizing agent, nor is it due to death and degenerative metabolites of the injected material.

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SPLENECTOMY EXERTS NO APPRECIABLE INFLUENCE UPON IMMUNITY AGAINST TRANSPLANTED TUMORS

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If the lymphocytes be the important agent in the production and distribution of immunity to transplanted tumors, as is asserted by Da Fano, Murphy, and others, it is not improbable that the spleen, a lymphocyte-forming organ, may take some part in the process. In an experiment previously reported (1), it was shown that the removal of the spleen of normal rats, either before or after inoculation with tumor, had no effect upon the growth energy, the percentage of takes, or the percentage of spontaneous absorption of the tumors. The present paper records additional experiments showing the lack of influence of the spleen upon certain phases of tumor growth and immunity.

Our first experiment confirms an observation of Brancati (2) that splenectomy does not influence the persistence of immunity in animals. Twenty-four rats bearing rat sarcoma 7, a tumor which is spontaneously absorbed, and which produces concomitant immunity in from 90 to 100 per cent of inoculated animals after three weeks' growth, were splenectomized one week after the complete absorption of their tumors. One week after operation, these animals and 24 control rats were inoculated with 0.003 gram of the same tumor strain. The operated animals showed 100 per cent immunity, while the 24 controls showed but 4 per cent.

The interval of one week between splenectomy and inoculation was arbitrarily chosen as a period of time sufficient for the effect of the operation to assert itself upon the organism, but insufficient for the complete assumption of the blood-forming function of the spleen by the bone marrow and lymph nodes.

That the removal of the spleen has no appreciable effect upon the fate of growths which eventually recede, is shown by the results of a second experiment. Forty-eight rats, inoculated with 0.003 gram of rat sarcoma 7, were divided into two groups at the end of twenty days' tumor growth, the period at which this neoplasm commences to regress. One group, consisting of 23 animals, was splenectomized, the other serving as controls. As is shown in chart 1, of the surviving spleen-free animals only one (rat 13) presented a tumor which showed continuous growth; in the other 14 animals and in all the control animals the tumors steadily regressed.

It has been stated (3) that splenectomized animals are more difficult to immunize than normal animals. The validity of this statement was tested in the following experiment. Mouse carcinoma 63 was the tumor employed, and mouse embryo emulsion was selected as the immunizing material and given in doses of 0.05 cc. A group of 72 adult mice was immunized, and on the day of immunization the spleens were removed from 24 of these animals. In order to control the effect of operation and anesthetic, the testes were removed from 24 others, while the third group of 24 animals served as nonoperated controls. Ten days later these three groups, and a control nonimmunized series of 24 mice, were inoculated with 0.003 gram of tumor 63. As will be noted in chart 2, 90 per cent of the spleen-free, and 87 per cent of the testes-free animals were immune, as compared with 96 per cent in the immunized controls, and 39 per cent in the nonimmunized controls.

The results of this experiment show that removal of the spleen or testes has little or no effect upon the immunity induced in adult mice by embryo emulsion.

The following experiments show that splenectomy does not render the body a more favorable soil for the growth of spontaneous carcinomata from other mice. Forty-three mice were splenectomized and from one to three days after operation were

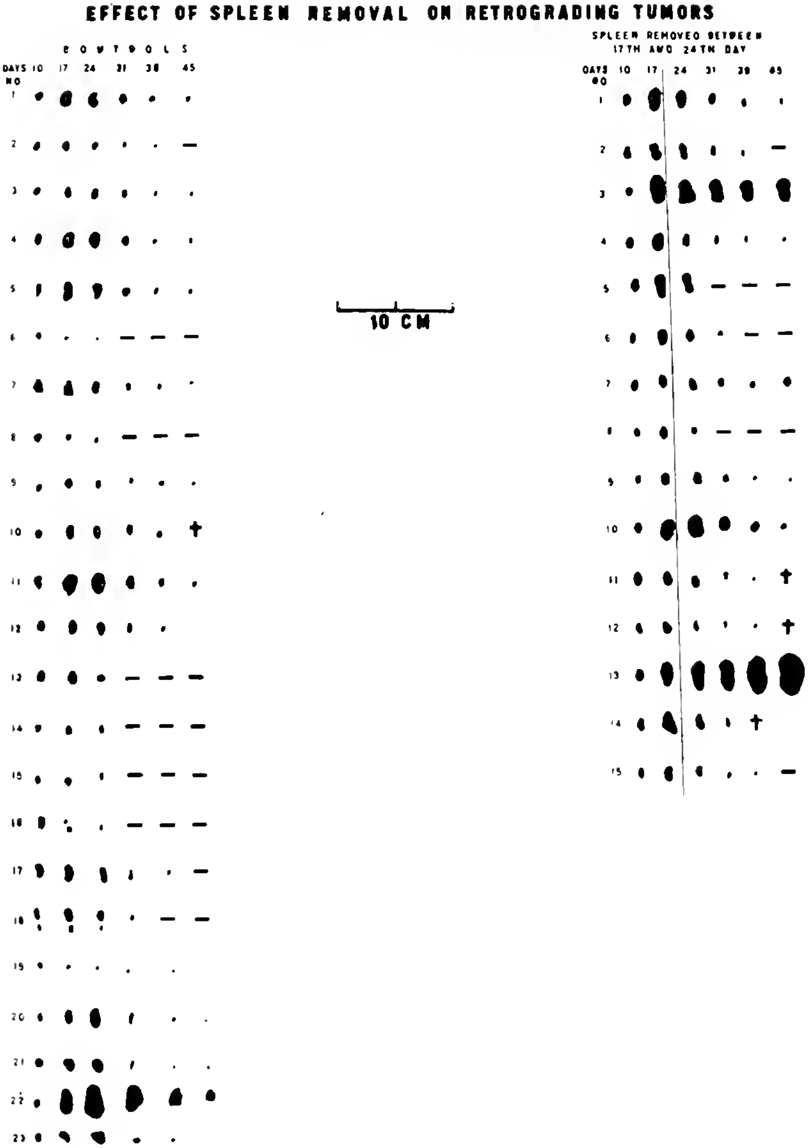
EFFECT OF SPLEEN REMOVAL ON RETROGRADING TUMORS
CONTROLS
DAYS 10 17 24 31 38 45
NO
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
10 CM
SPLEEN REMOVED BETWEEN 17TH AND 24TH DAY
DAYS 10 17 24 31 38 45
NO
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15

Chart 1

inoculated with spontaneous carcinoma 455, 50 control mice being inoculated at the same time with similar doses of the same tumor. Sixty days after inoculation, the control group showed one take, while the operated animals showed none.

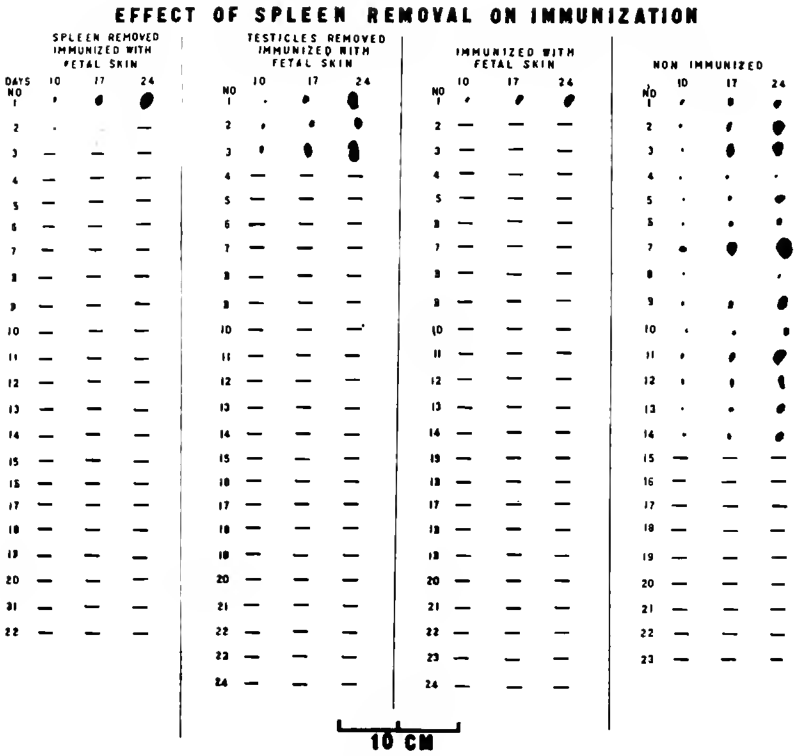

Chart 2

Forty other splenectomized mice were inoculated the day after operation with 0.003 gram of spontaneous mouse carcinoma 619, 48 nonoperated animals serving as controls. Sixty days after inoculation, there was one take in each of the two groups.

CONCLUSIONS

Splenectomy has no effect upon the persistence of immunity induced by the receding rat sarcoma 7.

Splenectomy does not influence the fate of regressive tumors.

Splenectomized animals are no more resistant to immunizing agents than are normal animals.

Splenectomy neither increases the percentage of takes nor favors the growth of spontaneous tumor grafts.

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LOSS OF THE POWER TO PRODUCE SARCOMATOUS TRANSFORMATION IN THE STROMA

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It will be assumed throughout this paper that a certain alteration which sometimes involves the stroma of carcinomata, and to which the term sarcomatous change has been applied by most authorities, is actually a sarcomatous transformation. A discussion of the reasons for provisionally accepting this opinion will be postponed, however, to a subsequent communication in which there will be adduced some new evidence which appears to support it.

Like some other attributes, such as keratinization, the power to induce sarcoma is not always a permanent possession of the carcinoma cell. This first came to light in the Flexner-Jobling tumor, an adenocarcinoma of the seminal vesicle of the rat, which between the fifth and eleventh generations cleared itself entirely of a sarcomatous portion (1). That was some eight or nine years ago, and the reappearance of sarcoma in this neoplasm has never been reported; but whether the power to produce sarcoma has been totally lost, or is still potentially present, only years of observation can decide.

Another tumor to lose the power of initiating sarcomatous change is an adenocarcinoma (no. 37)[1] of the mouse breast described by Bashford, Murray, and Haaland (2), and again in still greater detail by Haaland (3). In the most recent account of this growth, Bashford (4) wrote that in some of its strains the power had been lost, or was at least latent.

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[1] Albrecht and Hecht (Wien. klin. Wchnschr., 1909, xxii, 1739) mentioned a growth sent them by Bashford, which lost and regained the power to produce sarcoma, but did not say whether or not it was tumor 37.

A third example, reported by Stahr (5), was a fissure-forming carcinoma of the mouse, in which the carcinomatous moiety, after almost completely disappearing, emerged again as pure carcinoma in some of the animals; in others, it was still able to bring about the sarcomatous alteration.

Perhaps the most striking case of all is Russell's tumor (no. 100) (6), a hemorrhagic adenocarcinoma of the mouse mamma, which continued for about three years to produce sarcoma in practically every mouse in which it had grown for two months or so. Then, within the brief period of four months, one of the strains ceased to elicit this transformation even in tumors two hundred days old, and two others appeared to be rapidly losing their ability to bring it about.

Finally, Beneke (7) has recorded a human neoplasm in which the sarcomatous constituent appeared to have been lost. A fragment removed for diagnosis from a tumor of the upper jaw contained both carcinoma and sarcoma, though when the whole growth became available for examination a few weeks later, it was found to be pure carcinoma. The case is not conclusive, however, for the sarcomatous change may have been localized in and about the portion of the growth from which the first piece was removed. Furthermore, even though one could be sure that, contrary to what usually takes place in the mouse, the carcinomatous portion had suddenly overgrown and repressed the sarcomatous, the occurrence would not be entirely analogous to the more or less gradual loss of the power to produce sarcoma during repeated transplantation of a mouse tumor, and Beneke's case is therefore included only for the sake of completeness.

THE SPONTANEOUS TUMOR

The neoplasm (no. 48) to be described in this paper, the fifth mouse carcinoma to possess and lose the power of producing sarcoma in the host's connective tissue, was found in the left inguinal mamma of a female of unknown age. This growth, which measured 1.6 by 0.8 cm. and weighed 2.1 grams, was removed by operation on March 17, 1914, and inoculated into thirty-four young mice, in the course of an experiment quite unconnected with the present subject. The only tumor which developed in these animals proved to be a carcinosarcoma, and the disc which had been removed from the spontaneous growth for microscopic purposes was thereupon cut in serial sections. Nothing even suggesting sarcoma was found in this slice, which, however, represented no more than perhaps a quarter of the whole tumor. The neoplasm was an adenocarcinoma, differing from the ordinary spontaneous tumor of the mouse mamma only in being much more hemorrhagic and in having an extraordinarily scanty stroma.

THE DAUGHTER TUMORS

Of the thirty-four mice into which the spontaneous growth had been transplanted, twenty lived for more than two months, and one of these developed a tumor (generation 1), as has been said. This appeared a month after implantation and grew steadily until, on July 15, 1914, four months after inoculation, it had reached the size of 1 by 0.7 cm. On this date it was transplanted, giving generation 2A. The subsequent history of the tumor can be readily appreciated by reference to the chart (fig. 1). Thus, three growths from generation 2A were transplanted, giving rise to generations 3A, 3B, and 3C, and so on. Certain generations, however, used for experiments entirely unassociated with the subject now under discussion, have been omitted from the chart because a microscopical examination was not carried out, and the most recent generations have been excluded, also, because the tumors in them are, of course, still young. The growth is now, after about three years of cultivation, in its twenty-fourth generation; hence, each generation represents approximately a month and a half in time. The heavy figures (generations 1, 2A, 3A, 3B, and 4A) indicate the presence of one or more carcinosarcomata in a generation; the small figures imply pure carcinoma. The first generation to contain no mixed tumors (5A) was transplanted on December 3, 1914. The number of growths examined in each generation, with their several ages, may be found in a protocol at the end of the article.

It is unnecessary to enter into a minute discussion of the growth of tumor 48, inasmuch as the sarcomatous condition did not persist long enough for comparison to be instituted between the growth rate and the appearance of sarcoma. It will be sufficient to say that the percentage of successful inoculations has varied from nothing to one hundred, and that growth is fairly rapid and usually continuous, though spontaneous absorption is not at all rare.

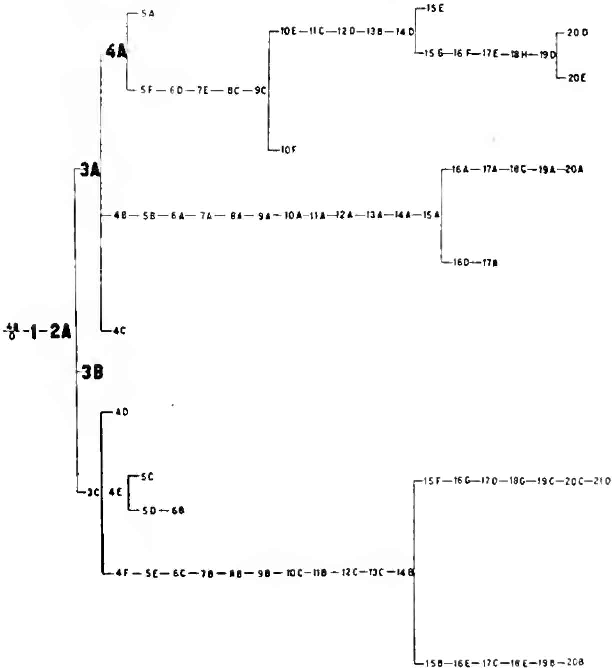
5A
4A
5F—6D—7E—8C—9C
10E—11C—12D—13B—14D
15E
15G—16F—17E—18H—19D
20D
20E
10F
3A
4B—5B—6A—7A—8A—9A—10A—11A—12A—13A—14A—15A
16A—17A—18C—19A—20A
16D—17B
4C
$\frac{48}{0}$-1-2A
3B
4D
3C
4E
5C
5D—6B
4F—5E—6C—7B—8B—9B—10C—11B—12C—13C—14B
15F—16G—17D—18G—19C—20C—21D
15B—16E—17C—18E—19B—20B

Fig. 1. Chart Showing the Various Generations of Tumor 48

HISTOLOGY OF THE DAUGHTER TUMORS

Except for the few examples of carcinosarcoma, tumor 48 has preserved its adenocarcinomatous type in the main up to the present time. Alveolar areas, however, are often encountered, and keratinization (fig. 2), which will be discussed in a later paragraph, occurs with relative frequency. The stroma, which is not especially cellular, except in the neighborhood of hemorrhages or where muscle is being invaded, is occasionally sclerotic; more often, however, it is similar in all respects to the stroma of any other propagable mouse carcinoma. In Haaland's case, the sarcomatous change appeared in connection with a sclerotic stroma, and such a relation has been observed by the present writer in other growths; but in tumor 48 the two conditions do not seem to be so intimately connected with one another.

Seven hundred and forty-six tumors in the twenty-one generations shown on the chart reached an age at which they might profitably be examined, and of these, five hundred and thirty-eight, ranging in age from seventeen to two hundred and thirty-one days, were prepared for histological purposes. In the earlier generations, almost every growth was examined, but later on only those over seventy-five, and still later, over one hundred days old, were preserved. Many of these tumors were more than two months old, the age at which sarcoma generally developed in Russell's case, yet, except for the generations mentioned, sarcoma was never found; indeed, the stroma was rarely cellular enough to make the diagnosis of pure carcinoma difficult in any way.

As not every growth was cut in serial sections, an occasional area of sarcoma may have been overlooked; yet this cannot have taken place with any great frequency, considering the uniform failure to find sarcoma in all generations after the fourth, and the exact point at which the sarcoma disappeared is, in any case, a relatively unimportant matter. The general statement undoubtedly holds true, that in earlier generations the parenchyma of tumor 48 had the power to induce sarcomatous change in the stroma, and that within a few generations this power disappeared.

There can be no doubt regarding the presence of sarcoma in the earlier generations, for the appearance of the affected tumors, as shown in figure 3, for example, was exactly similar to that described by other writers. Even the characteristic halos (fig. 4) were present, first described by Haaland and his colleagues as peculiar areolas of lightly stained cells encircling the carcinomatous elements, and regarded by these authors as probably sarcomatous in nature. It is interesting to note that, according to Haaland, a phenomenon resembling halo formation has been found in mixed tumors of the human subject also.

Reference has already been made to the sclerotic stroma, represented by Haaland as somewhat similar, in pronounced cases, to that associated with scirrhous carcinoma of the breast in the human subject. This was present in tumor 48 in generation 4B, which was used for transplantation into generation 5B, and in a tumor of this later generation also (fig. 5). But though the discs which had been preserved from these two tumors were cut in serial sections, no sarcoma could be found. The condition occurred in later tumors, also, in generations far removed from those showing sarcomatous transformation, and does not appear, therefore, to have the significance, in this growth at least, that it possessed in the case of Haaland's neoplasm.

The appearance of tumor 48, when it grows as a pure carcinoma, is shown in fig. 6, which reproduces a tumor from the second generation (2A). Of six growths in this series, four were mixed and two appeared to be pure carcinomata; but as the whole tumor was not cut in either of these two cases, sarcomatous change may have been overlooked; or it may be, on the other hand, that this neoplasm, like those of Haaland and some other investigators, and unlike Russell's, did not produce sarcoma in every animal bearing it. The age of the tumors, at any rate, does not enter here, for the two which appeared to be pure carcinomata were ninety-nine and one hundred and fifty-three days old respectively, while sarcoma had been produced by the seventy-fifth day in other growths belonging to this generation.

KERATINIZATION AND SARCOMA DEVELOPMENT

The presence of keratin in tumor 48, and in another mixed growth (1124) now under observation in this laboratory, led to a search of the literature to see whether any of the carcinosarcomata already reported in animals had contained keratin, as well as to a re-examination of the sixteen that have come under the observation of the writer. So far as the cases already described are concerned, only Lewin (8), among some dozen observers, records the presence of keratin; this was discovered in the third generation of a transplantable carcinoma of the rat, which in the fifth and subsequent generations induced the sarcomatous transformation in its stroma.

Any account of the sixteen mixed growths about to be discussed should, perhaps, be prefaced by recalling to the reader the fact that keratinizing carcinomata are growths in which the epithelial cells are often flattened out into a spindle shape, a source of diagnostic error mentioned by both Ribbert (9) and Henke (10) in their discussion of carcinosarcoma. But it may be asserted with some confidence that the sixteen tumors now under discussion include only such neoplasms as the majority of pathologists would unhesitatingly call true carcinosarcomata.

Of these sixteen mixed tumors, two belong to the Rockefeller Institute, the writer being indebted to the courtesy of Dr. Peyton Rous for the privilege of examining them; a third became available through the kindness of Dr. H. Gideon Wells of the Sprague Memorial Institute at the University of Chicago. To both of these gentlemen the writer wishes, therefore, to extend his thanks.

Ten spontaneous tumors, among the sixteen growths examined, contained keratin—T15412 (Dr. Wells), $\frac{370}{0}$, $\frac{797}{0}$, $\frac{1124}{0}$, $\frac{562}{0}$, $\frac{646}{0}$, $\frac{516}{0}$, $\frac{746}{0}$, $\frac{1077}{0}$, 125 (Dr. Rous). The first four had a moderate, the remainder a small amount.[2] Two of the ten had to be examined in serial sections before keratin could be found $\left(\frac{746}{0}, \frac{1077}{0}\right)$. An eleventh case is tumor 48, which forms the subject of this paper, and in which no keratin was demonstrable in the spontaneous growth, even in serial sections; it appeared first in the daughter tumors. In five spontaneous neoplasms—$\frac{590}{0}$, $\frac{484}{0}$, $\frac{961}{0}$, $\frac{172}{0}$, 62 (Dr Rous)—no keratin was discovered, though the first three were examined in serial sections. Still, as the entire tumor had not been preserved, only the disc fixed at autopsy was available for the inquiry; keratin may have been present, however, in some other part of the growth, or, as in the case of tumor 48, it may have existed potentially, and might have become manifest in daughter tumors, had these five neoplasms been propagated.

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[2] Where only a few points in the tumor were keratinized, the amount is said to have been small; where keratin was scattered throughout the section, the amount is described as moderate; where the tumor consisted almost entirely of keratin, keratinization is said to have been extensive.

To say that eleven out of sixteen carcinosarcomata contained keratin, however, means nothing unless one has some information regarding the occurrence of keratinization in those mammary carcinomata of the mouse which do not induce the sarcomatous change in their stroma. A thousand such spontaneous neoplasms were accordingly examined, though not in serial section, since it was felt that the labor and time necessary for such an inquiry would be out of all proportion great in comparison with the information acquired. Nevertheless, it may be assumed that the figures obtained represent with fair accuracy the proportion of keratinizing tumors, for the occurrence of this change was found to be approximately the same in most of the groups of one hundred into which the whole series was divided. Thus:

	Keratinizing tumors
The first 100 contained	20
The second 100 contained	24
The third 100 contained	23
The fourth 100 contained	20
The fifth 100 contained	30
The sixth 100 contained	22
The seventh 100 contained	17
The eighth 100 contained	22
The ninth 100 contained	22
The tenth 100 contained	28

In 1,000 tumors there were thus 228 containing keratin, or about 23 per cent.

Nineteen of these were extensively keratinized, forty contained a moderate amount of keratin, and one hundred and sixty-nine a slight amount. It was found to make no difference in the percentage of keratinizing growths, whether multiple tumors from the same animal were or were not included.

On the whole, then, it is probable that about one-quarter of all the spontaneous mammary carcinomata in the mouse produce more or less keratin, and if this figure be accurate, it might be expected that of sixteen such growths only four or so would exhibit this change; yet of the sixteen tumors which produced sarcoma, eleven, or about 69 per cent, were of the keratinizing type.

It is obvious that it would be unwise to generalize too freely from these figures. In thé first place, the number of cases investigated is entirely too small to admit the conclusion that a cancroid is more likely than other carcinomata to induce the sarcomatous transformation in its stroma. Secondly, had it been possible in every case to examine the whole tumor in serial sections, the figures just given might be profoundly altered. In the third place, some excellent pathological judgment still asserts that what is ordinarily called sarcomatous transformation is in reality but the assumption by the epithelium of a spindle shape; and it has already been indicated that the keratinizing carcinoma is the very neoplasm in which this is apt to occur. How difficult the diagnosis of sarcoma development may actually be, is evident from the fact that such an experienced observer as Apolant (11) was sometimes in doubt. Finally, only about 12 per cent of the cases of sarcoma development in man have involved keratinizing neoplasms; still, human mixed tumors are hardly comparable with those of the mouse, because they may occur in practically any organ of the body, while in the mouse carcinosarcomata are found almost or quite exclusively in the mammary gland.

For these reasons a definite assertion is quite impossible. One can merely raise the question whether keratinizing carcinomata may not, perhaps, induce the sarcomatous change in their stroma more frequently than do other neoplasms, and suggest that all carcinosarcomata discovered in the future be examined in reference to this query.

DISCUSSION

There are really two questions concerned in the abrupt disappearance of sarcoma from the later generations of tumor 48. First, the failure of any sarcomatous portions that may have been transplanted together with the carcinomatous, to grow in the new hosts, a circumstance observed by Haaland (loc. cit., p. 223), and secondly, a loss by the carcinoma of its power to produce sarcoma. As for the former, it is possible that none of the sarcomatous part of tumor 48 was ever transplanted, for every effort was made at first to select for propagation only the parenchyma, after the method of Haaland, in order that the carcinomatous portion might not be overgrown by the sarcoma and lost—a needless precaution, however, as subsequent events showed. Whether the first factor was or was not involved, the second most probably was.

This loss of the power to produce sarcoma, entirely unsuspected when carcinosarcomata in the mouse first came under investigation, is of some theoretical interest in view of the suggestion by Apolant (12), that the natural end of every carcinoma might be the initiation of sarcoma and, finally, suppression by this latter component, the cells of the carcinoma thus digging their own grave. This is undoubtedly the fate of a few carcinomata, but not by any means of all, as cases like the present one sufficiently indicate. A second aspect, of perhaps even greater importance, has been pointed out by Bashford (13) as follows:

> The attempt to explain sarcoma development by transference of a virus from the carcinoma cell to the connective tissue is unsatisfactory, since it will not explain the subsequent loss of this property and, at the same time, the continued growth by the carcinoma cells

Only years of observation will answer the question of this author (14), whether the power to produce sarcoma is a permanent quality, potentially present, at least, in the primary neoplasm and in all its daughter tumors, or a transitory attribute of certain carcinomatous parenchymata and a permanent property of others.

It is evident, in any case, that this power is subject to variations, both as to its intensity and its duration. Thus some parenchymata are able to initiate the sarcomatous transformation in almost every mouse, while others are not; and again, the same parenchyma may require but thirty-eight days in some generations, and more than seventy in others (Russell). Such variations as these are not due to individual differences in the hosts, for both Loeb (15) and Russell have shown that within any one generation the sarcomatous change sets in at the same time in all the mice. As to its duration, the power may persist for three years, as in Russell's tumor, or for about nine months, as in the neoplasm described in this paper.

SUMMARY

A transplantable mouse carcinoma, which exhibited in the first generation the power to cause sarcomatous transformation in its stroma, lost that power after the fourth generation, and has not regained it during three years' cultivation.

The question is raised whether mouse tumors containing keratin may not, perhaps, induce sarcoma development in their stroma more often than other types of carcinoma.

PROTOCOL

The following protocol gives the total number of tumors, in each generation, which reached an age sufficiently advanced for examination, the number of these examined, the results of this examination, and the age of the youngest and oldest tumors in each group.

The numerator of the fraction represents the number of tumors examined; the denominator represents the number in each series. Under the term "necrotic" are included those tumors in which degeneration was so far advanced that no judgment was possible and, for the sake of simplicity, those tumors, also, which by reason of the death of the animal were too decomposed for examination.

PROPORTION EXAMINED		AGE IN DAYS	CARCINOMA	CARCINOSARCOMA	NECROTIC
1	1/1	89		1	
2A	6/11	75–205	2	4	
3A	7/13	36–74	6	1	
3B	1/1	73		1	
3C	10/14	34–61	10		
4A	33/38	30–100	32	1	
4B	29/38	31–71	24		5
4C	14/24	53–84	13		1
4D	43/48	48–99	41		2
4E	31/33	32–97	29		2
4F	5/7	24–86	5		
5A	12/13	52–90	11		1
5B	5/7	30–83	5		
5C	12/14	45–70	10		2
5D	20/21	17–64	20		
5E	13/17	27–65	11		2
5F	9/13	30–122	8		1
6A	18/18	28–57	17		1
6B	14/15	37–105	13		1
6C	11/13	17–101	10		1
6D	5/7	35–124	4		1
7A	12/12	18–94	10		2
7B	7/8	29–85	6		1
7E	4/6	26–87	4		
8A	5/10	35–146	4		1
8B	4/4	32–74	3		1
8C	7/8	35–128	7		
9A	5/10	30–135	5		
9B	7/9	29–101	7		
9C	2/6	27	2		
10A	6/8	30–95	6		
10C	8/11	38–141	7		1
10E	1/1	25	1		
10F	1/1	45	1		
11A	2/3	25–87	2		
11B	10/13	31–151	9		1
11C	1/1	24	1		
12A	2/3	26–94	2		
12C	10/15	21–108	10		
12D	5/8	21–120	4		1
13A	2/3	26–70	2		
13B	3/8	32–99	3		
13C	7/10	25–231	7		

PROPORTION EXAMINED	AGE IN DAYS	CARCINOMA	CARCINOSARCOMA	NECROTIC
14A 3/6	27–52	3		
14B 9/14	25–166	9		
14D 3/10	24–82	3		
15A 7/11	26–89	7		
15B 7/8	34–132	7		
15E 1/1	100	1		
15F 21/32	33–153	18		3
15G 4/7	28–91	4		
16A 1/1	34	1		
16D 1/1	33	1		
16E 7/8	25–107	7		
16F 1/5	34	1		
16G 8/18	29–141	8		
17A 1/4	107	1		
17B 1/2	19	1		
17C 6/10	38–110	5		1
17D 3/11	31–112	3		
17E 5/6	31–112	5		
18C 5/12	35–112	5		
18E 7/13	37–101	7		
18G 2/5	48–81	2		
18H 1/1	42	1		
19A 2/4	34–93	2		
19B 4/5	35–104	4		
19C 1/3	42	1		
19D 2/2	42–82	2		
20A 3/5	31–112	3		
20B 5/10	64–71	5		
20C 1/1	42	1		
20D 1/1	40	1		
20E 1/1	34	1		
21O 4/4	40–128	4		

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PLATE 1

FIG. 2. KERATIN IN AN 82-DAY OLD TUMOR OF THE SIXTH GENERATION. × 200

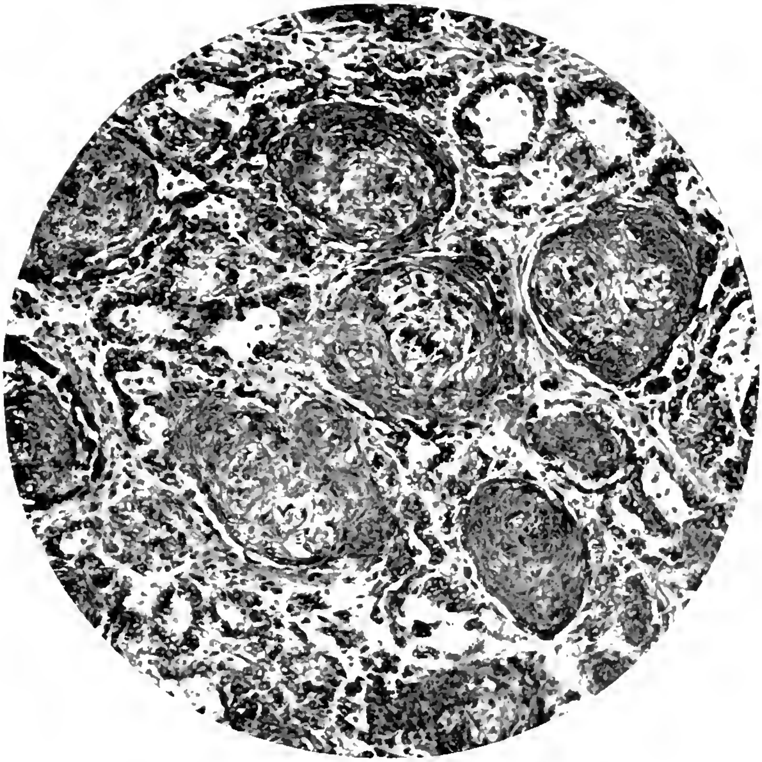

PLATE 2

Fig. 3. Sarcomatous Change in a 53-day Old Tumor of the Third (3A) Generation. × 200

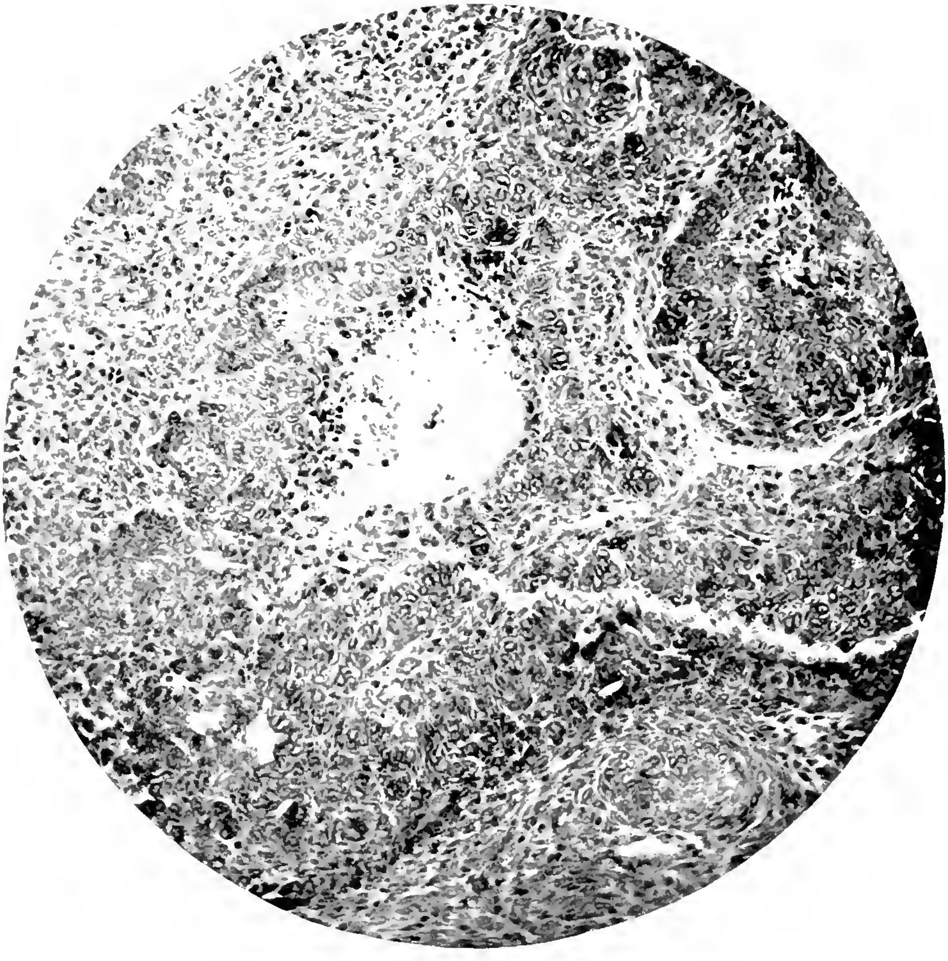

PLATE 3

FIG. 4. HALO IN A 73-DAY OLD TUMOR OF THE THIRD (3B) GENERATION. × 400

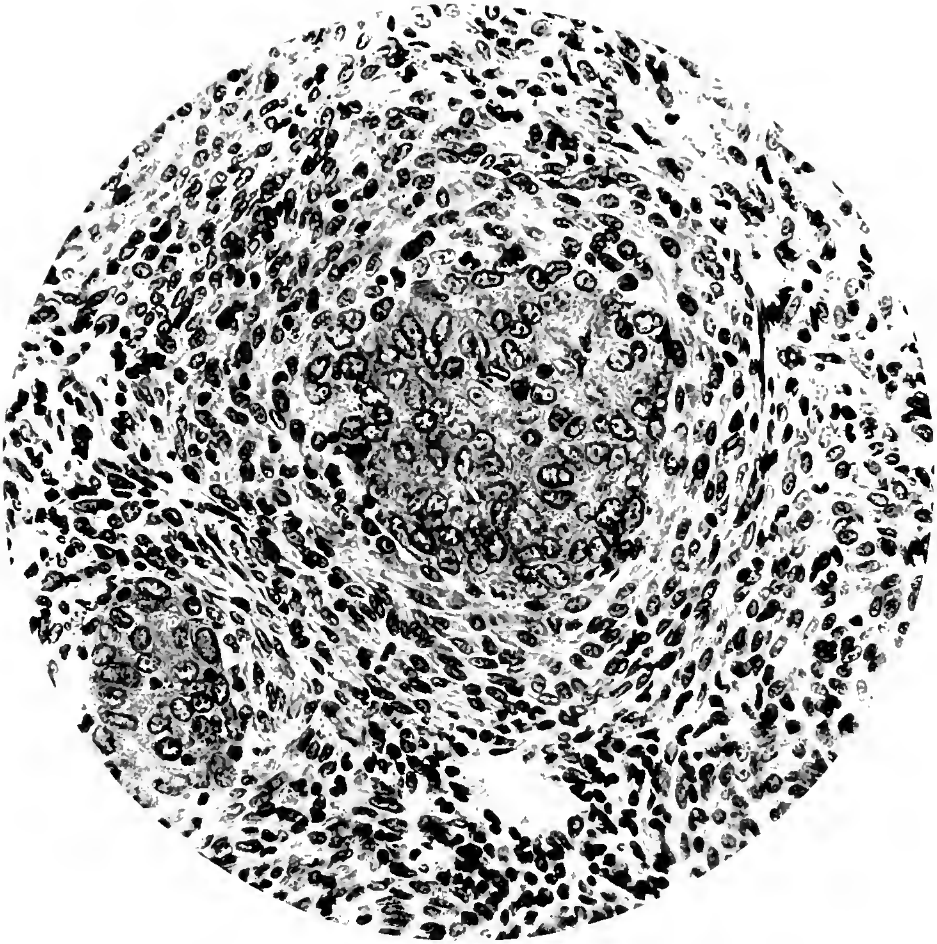

PLATE 4

FIG. 5. SCLEROTIC STROMA IN AN 80-DAY OLD TUMOR OF THE FIFTH GENERATION. × 100

FIG. 6. TUMOR 18 GROWING AS A PURE CARCINOMA. GENERATION 2A. × 200.

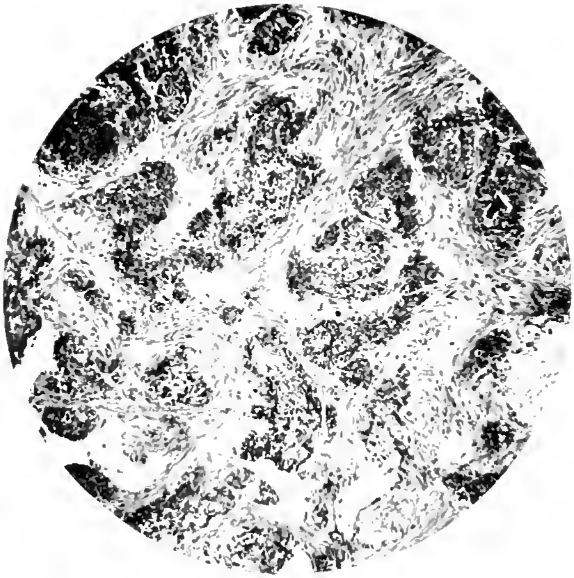

FIG. 5

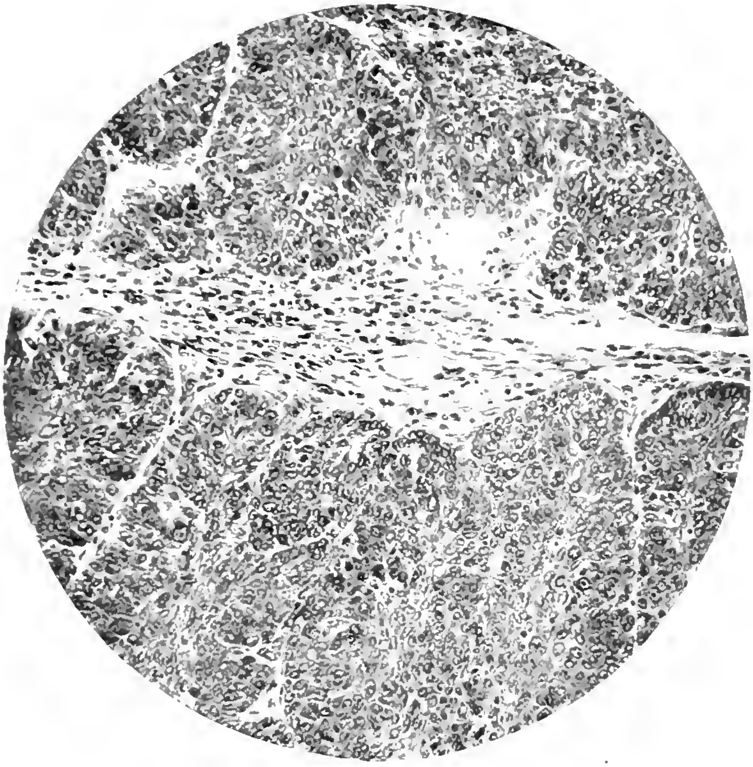

FIG. 6

PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH

TENTH ANNUAL MEETING

Held in New York, April 5, 1917

1. Report of the Council

The following members were present at this meeting; Dr. Harvey R. Gaylord, president; Dr. F. C. Wood, vice-president; Dr. Richard Weil, secretary and treasurer; Dr. James Ewing, Dr. H. Gideon Wells, Dr. J. B. Murphy. Absent, Dr. F. P. Gay.

The report of the treasurer, showing a balance on hand of $297.41, was read and accepted.

Dr. William H. Woglom, of the Crocker Research Fund, was elected as councillor to replace Dr. F. P. Gay, of the University of California, automatically retired by the time limit.

The following officers were elected for the ensuing year: Dr. F. C. Wood, president; Dr. Richard Weil, vice-president; Dr. William H. Woglom, secretary and treasurer.

The Council for the ensuing year consists of the above officers and Dr. Harvey R. Gaylord, Dr. James Ewing, Dr. H. G. Wells, and Dr. J. B. Murphy.

NEW MEMBERS

Active Member

Dr. Carlos Arroyo, New York

Associate Members

Dr. Martin J. Synnott, Montclair, N. J.
Dr. Barnet Joseph, New York
Dr. Douglas Quick, New York

Miss Harriet F. Holmes, of Chicago, was transferred from associate to active membership.

The resignations of the following members were accepted: Dr. C. L. Gibson, Dr. Arthur Holding, Dr. Frederick Jones, and Dr. J. Clarence Webster.

The following resolutions were passed:

1. That the secretary be instructed to prepare a printed list of members and of non-members subscribing to the Journal to be forwarded to the members of the Society with the request that their efforts be used to increase the membership.
2. That the treasurer be instructed to pay to Williams and Wilkins, for the

Society, the sum of two hundred and fifty dollars ($250) out of the treasury of the Society;

That the publishers be requested, in accordance with the letter of Mr. Passano of February 23, 1917, to carry the balance of the deficit to the account of the Journal;

That the secretary be instructed to enter a protest against the expensive character of the solicitation campaign, and to state that the Association reserves the right to decline joint responsibility for any outlays for solicitation in future years, unless the consent of their treasurer thereto be previously secured.

3. That the present editorial staff be continued for the coming year.
4. That Dr. Wells be instructed to consider with the American Association of Pathologists and Bacteriologists, and the American Association of Immunologists, a plan for future federation to embrace those associations and ours.

Addendum: The members of the Council informally pledged themselves to raise fifty dollars ($50) each toward the deficit of the Journal.

2. SPONTANEOUS MALIGNANT TUMORS OF THE ALIMENTARY CANAL IN MICE

Dr. Maud Slye, Miss Harriet F. Holmes, and *Dr. H. Gideon Wells* (Chicago):

SUMMARY

After discussing the relative infrequence of cancer of the stomach in all animals except man, the authors described the primary tumors found in the alimentary canal among 16,000 mice of all ages, dying natural deaths, and thoroughly examined at autopsy. There were found two squamous cell carcinomas of the cardiac portion of the stomach, one with metastases in the mesentery; also a typical cylindrical cell, tubular carcinoma of the pylorus, apparently resulting from chronic irritation produced by a hair ball in the stomach, and with metastasis in the regional lymph nodes.

A growth resembling closely a sarcoma of the stomach was also described. No tumors were found in the intestines except an early squamous cell carcinoma arising on the external surface of the prolapsed rectum, which had been under observation for four months before the death of the mouse. All four carcinomas were in old males.

(The full report will be published in the Journal of Cancer Research for July, 1917).

DISCUSSION

Dr. Wm. H. Woglom (New York): To Dr. Wells' three cases I should like to add one recently reported at the New York Pathological Society by Dr. Itami. It was exactly similar to the first two described by Dr. Wells, and I mention it only on account of the rarity of such tumors.

Dr. W. C. MacCarty (Rochester, Minn.): Were these growths associated in any way with ulceration?

Dr. Wells: Yes; in the case of the mouse that had the hair ball there was a typical chronic inflammatory ulcer. The others occurring in the squamous portion had secondary ulceration, but I have never seen anything in mice resembling the round ulcer in man. Dogs, I understand, have ulcer of the stomach very frequently, yet they do not have gastric carcinoma.

Dr. MacCarty: Out of 1000 cases of carcinoma of the stomach in man, a condition apparently the same occurred ten times. It takes some time fully to prove itself carcinoma, and it is a very difficult thing to diagnose.

3. A Study of Trauma in Relation to Primary Mouse Tumors

Mr. M. C. Marsh (Buffalo):

SUMMARY

Acarine ectoparasites of mice were introduced into these animals both by feeding and by inoculation, in an endeavor to produce neoplasms; the few tumors which occurred, however, were no more attributable to the parasites than to independent trauma. Many other experimental procedures, involving trauma with and without mites, resulted negatively or nearly so. In a third series of experiments, finely pointed pipettes or needles carrying a current of air were introduced into the nipples of mice and the air was deposited under the skin and occasionally in the mamma. This definite trauma in the presence of air was followed by an incidence of epithelial mammary tumors reaching 80 per cent among 11 old breeders during the succeeding six months. The maximum expectancy among similar small lots of normal mice in which tumor ancestry was irregularly distributed was shown by controls to be between 35 per cent and 40 per cent. Virgin mice did not react to the same procedure, and primiparous mice were intermediate.

DISCUSSION

Dr. Wells: Stock raisers use this method of injecting air into the lacteal ducts to cure certain diseases of cows. Tumors of the mammary gland are rare in cows ordinarily; does Mr. Marsh know whether neoplasms have resulted from this injection of air in cows?

Mr. Marsh: I have heard of that procedure, but I don't know of any results that have come from its use.

Dr. Woglom: So far as our knowledge goes at present, cancer has three causes: Age, chronic irritation and a certain hereditary predisposition. Most of the attempts to produce cancer by chronic irritation have been carried out in young animals, where only one (chronic irritation) of these three causes was present. In those where old animals have been employed, two (age and chronic irritation) existed. Some years ago I began a series of experiments on mice already the subject of spontaneous carcinoma, in which all three of the factors were therefore at hand (age, disposition, and irritation). Though here it was necessary only to find the appropriate irritant, this has not yet been discovered; no carcinoma has appeared at the site chosen for injury. Mr. Marsh has displayed great ingenuity in gaining entrance to the mammary ducts. I succeeded occasionally in aspirating various substances into the enlarged passages of pregnant mice by suction applied to the internal surface of the mamma after a flap of skin had been reflected, but the result was always uncertain and the experiment, as has already been stated, never gave rise to a tumor.

Dr. Richard Weil (New York): As the discussion is along the lines of the factors contributory to cancer, I may, perhaps, add a suggestion. Dr. Woglom has certainly described the most important of these factors. It seems to me, however, that we must also recognize as one of them the disturbance of internal equilibrium; according to the work of Loeb, the removal of the ovaries and the prevention of conception produce conditions which have a definite, though negative, influence on the occurrence of mammary tumors. There is an observation, too, which I have made, which seems to fall in the same group, although it also involves an hereditary factor. I have for some years had occasion to examine a large number of guinea-pig ovaries, taken at random from normal animals, and have found these organs practically normal on gross inspection. More recently, however, I have been examining guinea-pigs from a special strain cultivated by Dr. Stockard, a strain of which the ancestry is definitely known to contain a number of individuals which had been exposed to the fumes of alcohol over a considerable period. The effect of this treatment may manifest itself in the second or third generation by the production of monstrosities. In studying the ovaries of guinea-pigs with such ancestry, striking changes were found in a very large proportion of the animals. These consist in the presence of a high degree of cystic degeneration, often resulting in marked enlargement of the organ. Microscopically, there were found epithelial changes, not indeed malignant, but certainly they could be called pre-cancerous. I am not even intimating that these cysts are true tumors, but I think that the observation is nevertheless extremely suggestive. It makes one suspect that chemical trauma to the ancestry may make its effects apparent in later generations in the form of epithelial degeneration, or aberration.

Dr. Ellis Kellert (Albany): In connection with Mr. Marsh's paper, I should like to speak of a kidney received at the Bender Laboratory after its removal by Dr. J. A. Sampson. The patient was a married woman, aged 54, who had had symptoms of renal calculus leading back eight years, though during the past year the symptoms have become much more pronounced. The kidney (right) was greatly enlarged and contained two large, irregularly shaped calculi. Occupying its pelvis and involving the calices and pyramids, there was found a large, infiltrating, tumor which proved microscopically to be an epidermoid carcinoma of that type frequently found in the bladder.

Dr. Harvey R. Gaylord (Buffalo): I should like to make a comment upon Mr. Marsh's work. The character and delicacy of his tests place his work in a category by itself, for one can imagine the patience and perseverance needed to inject anything into the nipple of a mouse. Many investigators have tried to reach the mammary gland without success—it is a very natural idea—but Mr. Marsh's patience has conquered the difficulty. In the 11 mice reported, the results certainly were very striking. It is highly suggestive work and correlates many of our ideas. The first evidence of carcinoma, as pointed out by Apolant, is found in the group of cells in contact with the duct, suggesting as a cause something entering the gland from outside, and it would appear that Mr. Marsh has discovered a technic for investigating the etiology of breast tumors in the mouse, that will yield interesting results. Another factor of great importance is this, that experimenters should know the kind of stock that they are using, for there is great necessity for proper controls in experiments of this kind, and only when experiments are properly controlled and checked up can we feel that we are getting on firmer ground. I should like to know how many cases Dr. Woglom handled.

Dr. Woglom: Between 75 and 100. I should be glad to go over these cases with Mr. Marsh after the meeting.

Mr. Marsh: I have tried injection of different solutions and I have tried injecting water. It is difficult enough to inject air, but more difficult to inject water. The pipette has to be big enough to carry a stream and that makes it too big to enter the nipple properly. The injection of air, besides being feasible (although it costs trouble and mice) is very simple, and it is a process, furthermore, to which mice are more or less exposed in conjunction with injury. Injuries often carry air into the gland. Thus, when I implanted mites under the skin of mice, it appeared that the results might be attributed to air plus the injury; that was how the idea of air plus injury was developed. In a quantitative sense I have accomplished little, but in a qualitative sense I think important results are involved.

4. The Spleen Exerts no Influence upon the Growth of Transplanted Tumors

Dr. Dudley H. Morris (New York):

SUMMARY

Numerous attempts have been made to demonstrate the influence of lymphoid tissue, especially spleen and bone-marrow, in resistance to transplanted tumors. The specific effect of the spleen in immunity has been repeatedly asserted and denied, but most of the experiments on splenectomized animals are not convincing, as they have been done on such small series of animals as to render their conclusions insecure. For when a small number of animals is used, individual variations in resistance to tumor growth are so great as to make definite deductions unsafe.

To test the effect of splenectomy on the rate of growth of transplanted tumors, a large group (300 animals) was employed, of which 150 were splenectomized eight days after inoculation with the Ehrlich sarcoma. The tumors were regularly charted and observed for upwards of one month after inoculation. No difference was found in the two sets of tumors. Splenectomy practiced at other intervals before and after inoculation likewise showed no effect on the growth of the tumors.

5. Unsuccessful Attempts to Cause Recession of Growing Tumors

Dr. Dudley H. Morris:

SUMMARY

The attempt was made to cause growing tumors to recede by the subcutaneous injection or transfusion of lymphoid tissue emulsions (spleen and blood), obtained (1) from normal animals, (2) from animals with growing tumors, (3) from animals repeatedly negative to tumor inoculation, (4) from animals treated with repeated emulsions of the same variety of tumor, and (5) from animals receiving an immunizing dose of splenic tissue.

In each experiment, 24 animals with growing tumors received the injections of lymphoid tissue, and a similar number of control animals was kept for comparison. Doses of 0.1 to 0.3 cc. of spleen, and of 0.5 cc. of blood were used, and four to six or more injections given. The outcome of this series of experiments was completely negative. The injections did not influence in the slightest degree the progressive growth of the tumors.

DISCUSSION

Dr. F. C. Wood (New York): There is an enormous amount of labor required in the preparation of papers of this type, which may be described as street-sweeping papers, labor which is entirely disproportionate to the results obtained. This report is negative, but the literature is full of positive though contradictory statements, and until these are either controverted or confirmed by experiments carried out on a large scale, erroneous ideas will prevail. An example of this may be found in the fact that certain recent workers, who have felt that there is some correlation between tumor immunity and an increase in lymphocytes, have cited Braunstein's results as confirming their own, paying no attention to published reports of equally good workers on the absence of any effect upon growing tumors after the injection of large quantities of lymphocytic tissue.

Dr. Wells: In relation to the influence of lymphocytes on neoplasms, I have noticed that we find mice showing a condition like human lymphatic leukemia, with tremendous lymphocytosis, and nevertheless exhibiting various types of malignant tumors. These tumors grow as well in mice with, as in those without, leukemia. Of course, it is possible that these may not be normal lymphocytes, yet it is certain that a mouse may have a blood count of 500,000 leucocytes and nevertheless be the subject of a malignant tumor.

Dr. Gaylord: Dr. Wood speaks of these experiments as if they were final solely because they cover a large amount of material. There are other factors, however, more important than the mere question of numbers. Thus we have seen conflicting experiments in different laboratories employing the same technic and even the same tumor (Flexner-Jobling); for example, a series of published experiments indicating the sensitization of animals was repeated at Buffalo in exactly the same way, and opposite results were obtained; the behavior of this tumor in our hands differed from its behavior with other investigators. Dr. Morris' work is valuable because of the large number of animals, but we must realize the importance of changing conditions; the same strain of mouse tumor does not act in the same way at all times. A complex biological problem is involved here, and conflicting results are often obtained because the conditions cannot be exactly reproduced.

Dr. Weil: Has Dr. Morris made a study of the lymphoid tissue throughout the body in these cases? I do not know of any such observation, but it would be of interest to learn whether there is any reaction in these tissues which would differentiate or characterize animals with regressing tumors. One point that is difficult to explain is the fact that tumors which recede, always, so far as I know, show the first evidence of retrogression in the center, and not at the periphery;

yet the blood supply is richest at the periphery, while in the center it appears to be defective. I base this statement on the microscopic appearance, and not on injection preparations which, I believe, give fallacious results. If there were essential factors in the blood which produce the death and absorption of tumors, ought not evidences of regression to appear first in the areas most exposed to those destructive agencies? So far as I know, it is almost universally true that tumors show the reverse phenomenon, the necrosis being most marked at the center, where the action of these hypothetical agents is quantitatively least.

Dr. Morris: The objection has been raised that retrogression seems to proceed from within outward and that this is an argument in favor of the view that nothing in the blood is operable in the production of immunity. We have, however, to assume that there is in the body some distributing factor which conveys immunity from one site to another, otherwise it would remain local. The blood is the one factor which pervades the entire body. Just what the immunizing substance is, I do not pretend to say. The central recession of a tumor may mean simply that the parts where the blood supply is poorer are more vulnerable to the factor which is operable in immunity, since the general nutrition of the central area suffers first. As to the effect of the growth of tumors on the lymphoid tissue of the body, I have never observed any consistent changes attributable to tumor growth. I have not studied the question, however, at great length.

Dr. Weil: Might I make another statement? I think it is important to emphasize the fact that there is no necessity to assume a distributing factor which carries immunity throughout the body. This is really fundamental. We have merely to assume a distributing factor for the antigenic substance. If we assume that an inoculated tumor is in part absorbed into the circulation, it would act as a foreign protein, stimulating all the cells of the body to reaction. Under such circumstances a generalized immunity would result without the intervention of any circulating antibodies. All we have to assume, then, is a substance which will stimulate a generalized cellular immunity, as the basis of distinction between histogenic and humeral immunity.

Dr. Leo Loeb (St. Louis): The question raised by Dr. Weil might be answered in the following way: Specific toxic substances circulating in an organism are liable to attack peripheral as well as centrally located cells in the tumor. Both of these they weaken, but the peripherally situated elements, which are better nourished, can withstand the injurious effect, while the central cells, which are at a disadvantage as far as nourishment is concerned, succumb first. In the degeneration of deciduomata, which takes place whenever the corpus luteum ceases to secrete a chemical growth stimulus, there occurs likewise a primary degeneration of the central parts. In this case we may be sure that a substance which has control over the life and growth of the cells circulates in the vessels. Here again secondary factors of nutrition determine the place of initial necrosis. The fact that the central rather than the peripheral tumor cells degenerate first does not, therefore, speak against the presence of immune substances in the circulation.

As to the question whether the cells concerned in the destruction of tumors in immune animals are real lymphocytes or connective tissue elements, the following fact seems to point strongly to the conclusion that the small round cell infiltration in tumor and tissue immunity is due to the collection of lymphocytes around the tumor or transplanted tissue. In pigeons (and other birds) the proportion of lymphocytes in the blood is much greater than in the guinea-pig; correspondingly, in the case of homoiotransplantation of normal tissues there is a much more distinct round cell infiltration in the pigeon than in the guinea-pig.

Dr. Morris: In regard to a distributing factor, when immunizing material is injected subcutaneously, immunity develops to a certain extent and is conveyed throughout the body. While we have no definite proof that the blood conveys this, the blood is the most probable factor.

6. THE SIZE OF THE SPLEEN IN MICE IMMUNE TO TRANSPLANTABLE TUMORS

Dr. Wm. H. Woglom:

SUMMARY

The spleens of a large number of mice were measured at laparotomy. The mice were subsequently immunized, their immunity proved by tumor inoculation, and the spleens measured again several weeks later at autopsy. It appears probable that there is no relation between immunity and the size of the spleen, for this organ in immune mice may be either enlarged or of normal size. Conversely, mice known to have enlarged spleens are not resistant to tumor inoculation. The experiments, however, are still unfinished, and one does not desire to make too definite an assertion.

DISCUSSION

Dr. James B. Murphy (New York): We have attempted to study just this problem of the size of the spleen, and appreciate the difficulty of establishing a normal figure. We tried to measure this organ, and found that if the mouse is shaved, a rough estimate of its size can be obtained. This is not accurate enough, however, and we have recently removed a large number of normal spleens to establish a normal weight. In animals from one source, the average weight for groups of ten was fairly constant. Now we are engaged in removing the spleen in various stages of immunity and comparing the weight with the figures from normal animals. Apparently the spleens from immunized animals before the tumor is injected show the highest figures. But they are only slightly higher than the average. It seems to me well worth while to follow out this point. Anybody who has examined spleens will know that the thickness of the spleen plays a great rôle; it is thick in one place and thin in another. We shall have to resort to weight to determine any variation.

Dr. Wells: I can think of nothing that I should be less willing to base a statement upon, than the size of the spleen in mice; there is an almost unbelievable difference in their volume. The increase in weight of the spleen may be dependent upon different factors; thus it may have more blood in it, it may have an increase in its endothelial or lymphoid cells, or its size may be modified by its fibro-muscular tissue. Each factor may act from entirely independent causes, and hence determinations of the size of the spleen must take into account so many factors that unless the studies are made over many years on many millions of spleens, it is doubtful if reliable results could be obtained.

Dr. James Ewing (New York): The observations of pathologists on autopsy material have not demonstrated any uniform relation between the size and condition of the spleen and the presence of cancer in the human body. In general, cancer is a disease of elderly subjects, and the lymphoid tissues and spleen therefore, might reasonably be expected to show some physiological decline as compared with youthful subjects. It is an established observation of oncology that lymphocytes very often gather in large numbers about regressing foci of cancer cells, whereas very malignant and rapidly extending growths usually fail to show lymphocytic reaction. This is commonly interpreted as favoring the view that lymphocytes are an important factor in the tissue resistance to carcinoma. It may, however, be a secondary phenomenon.

Dr. Woglom: I quite agree with Dr. Murphy. One cannot tell the size of the spleen by observation through the shaved skin. I tried this method myself, and found an error of from fifty to one hundred percent when the findings were controlled by an autopsy performed immediately afterward. Hence it was necessary to open the abdomen in every case and measure three dimensions of the organ. It is obvious that in these experiments the spleens could not have been weighed. In reply to Dr. Ewing, may I say that the experiments have been carefully controlled? All the immunized mice were tested for the degree of immunity, and a full report of the work will be given in the paper soon to be published.

Dr. Ewing: How long did you observe the animals?

Dr. Woglom: Some were under observation for as long as three months. In declining to compare these findings with those in man, Dr. Ewing has in mind the fact that an animal cannot be immunized against its own tumor. For this reason one would not think for a moment of applying to man the results obtained in animals bearing transplanted tumors. The two types of neoplasm—spontaneous and transplanted—must always be sharply distinguished from one another.

7. THE QUESTION OF VIRULENCE OR ADAPTION

Dr. Wm. H. Woglom:

SUMMARY

When multiple (from 2 to 5) spontaneous tumors from the same mouse are transplanted, they usually give approximately the same percentage of success. Occasionally, however, there is a wide variation, as, for example, in the three tumors X, W, and V of mouse $\underline{8}\,\frac{2}{0}\,\underline{7}$ (lantern slide). Tumor X gave 16 per cent of pinhead-size receding growths (15 tumors among 94 mice); tumor W produced 55 per cent (60 tumors among 109 mice) of pea-size receding nodules, and tumor V gave 78 per cent (100 tumors among 128 mice) of progressively growing tumors about the size of a walnut.

If growth after transplantation were merely a question of the power of cells from a given mouse to adapt themselves to a new soil, multiple tumors from the same animal should be transplantable with equal readiness; and they usually are. But instances like the one just cited prove that in a few cases multiple growths from the same mouse give different percentages of success, and it may therefore be assumed that the growth energy ("virulence") exhibited after transplantation in such cases is not the same in all the tumors. As the experiments are still unfinished, however, this conclusion cannot yet be definitely asserted.

8. THE MORPHOLOGICAL APPEARANCE OF CANCER CLINICALLY CURED BY MEANS OF RADIUM AND ROENTGEN RAYS

Dr. I. Levin and *Dr. B. Joseph* (New York):

SUMMARY

An analysis of operative statistics demonstrates that in the great majority of cases of carcinoma the disease recurs sooner or later, notwithstanding the most radical operative procedures. Therefore temporary disappearance of the primary tumor, diminution in its size, cessation of clinical symptoms, and prolongation of life may be considered merely a clinical cure of the disease. Usually, as shown in previous publications of Levin, this clinical cure is accompanied by morphological changes, consisting in degeneration and ultimate destruction of the carcinoma cells, and the formation of dense connective tissue.

The present study is based on a series of cases which show undoubtedly a clinical cure, though no appreciable morphological change was found in any of the tumors. Apparently there has taken place in these cases an inhibition of the proliferating power of the carcinoma cells without any anatomical changes.

DISCUSSION

Dr. Loeb: I should like to ask Dr. Levin a question in regard to the statistics. Did these patients all die of cancer, or some from other diseases?

Dr. Levin: Only those cases are quoted in which the disease recurred.

9. THE EFFECT OF X-RAY ON IMMUNITY TO TRANSPLANTED CANCER IN MICE

Dr. James B. Murphy:

SUMMARY

Dr. Morton and I showed, two years ago, that there is an actual increase in the lymphocytes of mice which have been immunized and then inoculated with a transplantable carcinoma. If, however, animals are exposed to a series of small doses of *x*-ray, between the immunizing injection and the cancer inoculation, the immunity is neutralized.

Percentage of takes from cancer inoculation

	Experiment I	Experiment II	Experiment III	Experiment IV
Normals	60	88	88	80
Immunized	0	33	11	39
Immunized and *x*-rayed	40	100	88	86

Furthermore, normal rats and mice *x*-rayed in the same fashion show a higher per cent of takes, when inoculated with one of the transplantable tumors, than do untreated animals similarly inoculated.

The resistance to transplanted tumors resulting from a previous homologous tissue injection has been termed a *pan-immunity*, because it is effective against a variety of tumors as well as against grafts of normal tissues. Therefore it may be considered, in a sense, a non-specific reaction for a definite tumor. The results which we wish to report deal with the effect of *x*-ray on this resistance after it may be regarded as a more specific reaction.

Mice were first immunized and then, ten days later, inoculated with a carcinoma. Three weeks later, the animals which had proved resistant were divided into two equal lots, one being given a series of small doses of x-ray. After this, all the mice were inoculated a second time with the same strain of tumor. In the x-rayed series, there was about 90 per cent takes from the second inoculation, while in the non-x-rayed lot there was less than 10 per cent of tumors.

But a still more rigorous test has been made. A series of mice, proved immune to a specific tumor, was inoculated a second time with the same tumor, some three weeks after the first inoculation; practically all proved resistant to the second inoculation. Half of these animals were then given x-ray by the same method as in the first experiment, and then both lots were inoculated a third time with the same growth. Here again the x-rayed animals gave a high per cent of takes, while the others were in general uniformly immune to the inoculation.

Whether or not these results can be explained by the destruction of the lymphoid tissue alone, or whether we must look for some other reaction which runs parallel, it is impossible to say at the present time. In this connection, it might be mentioned that the x-ray, in the dose we have employed, has no appreciable effect on the formation of antibodies to bacteria.

This presentation is in the form of a mere preliminary note, as more numerous and complete experiments are in progress at the present time.

DISCUSSION

Dr. Ewing: I should like to ask the details of technic in the x-ray treatment. How much was administered, and for what length of time? I should like to know, also, what effect the x-ray had on the animals as a whole; small animals, it is known, are very susceptible to x-ray treatment. Were the animals exposed long enough to be fatally injured, and what rôle did this play in regard to the lymphocytes? Whatever the interpretation may be, it bears upon the application of the x-ray to the human subject; for some investigators believe that the whole body should be exposed to a small dosage, while others assert that the body as a whole should be protected by every known method, and only the tumor exposed.

Dr. Levin: In connection with Dr. Ewing's question, I should like to know how many of the x-rayed animals, in the experience of Dr. Murphy, died from the effects of the x-ray? I have found that rats frequently die soon after the radiation.

Dr. Murphy: The technic I have worked out is one by which we can x-ray these animals without affecting their general health. We have kept some animals over a year after such treatment, yet they showed no bad effects. When we gave one large dose, we obtained the results that Dr. Levin described; we now, therefore, give very small doses, repeated each day, mice receiving 7 small doses (10 amperes, 5.6 cm. spark gap, at 30 cm. distance, for two to three minutes). This can be carried on without injuring the health of the animals, and seems to cause a minimum damage to other than lymphoid tissue.

10. Preliminary Note on the Possible Effects of the Nervous System upon the Growth and Development of Tumors

Dr. I. Adler and *Dr. M. J. Sittenfield* (New York):

SUMMARY

It is generally conceded that tumors, both malignant and non-malignant, are not possessed of an innervation like that recognized normally in every tissue and organ of the body, such nervous elements as have here and there been found in them appearing to have no definite and functional relations to the general nervous system. It is even doubtful if the blood-vessels of tumors are subject to nerve control.

It has been suggested that this lack of nerve control might in some way be a causal factor in the autonomy and unlimited proliferation of tumors. As a preliminary test of this hypothesis, fifty male rats were inoculated in the testis with the Flexner-Jobling tumor; in half the animals, the testis was stripped of all its nerves, while in the other half the testis remained undisturbed, except for the tumor inoculation. After excluding from the series all those animals that had been killed and eaten, or were decomposed so completely as not to permit any decision, there remained fourteen inoculations in denervated testes, and twenty controls. All fourteen showed unusually large and rapidly growing tumors. Of the twenty controls, on the contrary, one had a small tumor in the omentum, with a perfectly sound testis, a second exhibited a very insignificant growth, and a third had a white patch which turned out to be a purely inflammatory lesion. Of the other seventeen controls, not a single one showed the slightest sign of tumor formation. The investigation is being continued.

DISCUSSION

Dr. Woglom: I am greatly interested in the negative results obtained by Dr. Adler and Dr. Sittenfield after the inoculation of the Flexner-Jobling tumor into the testis of untreated rats, because I have recently published a series of experiments which show that the tumor with which they worked can be readily inoculated into this organ under normal conditions.

11. The Significance of the Lymphocyte in Immunity to Cancer

Dr. M. J. Sittenfield (New York):

SUMMARY

The presence or absence of small lymphocytes around a cancer implant, or in the circulating blood, has been considered an essential factor in tumor immunity. A small or stimulating dose of x-ray is said to protect against tumor inoculation, while, on the other hand, the repeated administration of x-ray, by suppressing the activity of the lymphoid apparatus, is said to render an immune animal susceptible to transplantation.

In the experiments about to be described, however, when a high lymphocytosis was produced in rats by the injection of pilocarpine, and the animals were inoculated with the Flexner-Jobling adenocarcinoma, the number of takes was the same as in untreated controls.

In a second experiment, a group of normal rats was injected intravenously with leucocytic cream obtained from rats in which a high lymphocyte count had been induced by exposing them to the x-ray. This leucocytic cream, in turn, caused the small lymphocytes to rise to 25 per cent or more. When the Flexner-Jobling tumor was inoculated subsequently, however, the same percentage of takes was obtained as in the controls.

Immune rats, repeatedly inoculated in vain with the Flexner-Jobling tumor, were x-rayed several times, their lymphocytes undergoing in consequence a reduction to 7–4 per cent. Subsequent implantation proved that this immunity had not been abrogated, all the animals having maintained their resistance to this growth.

In brief, the production of a high lymphocytosis in the first two instances, and the inhibition of the lymphoid apparatus in the third, did not affect either immunity or susceptibility, and it may, therefore, be regarded as questionable whether the lymphocyte per se acts either protectively or destructively in cancer immunity.

DISCUSSION

Dr. Ewing: It is quite evident that Dr. Sittenfield and Dr. Murphy have been using different forms of radiant energy. Perhaps their divergent results may be explained on this ground.

Dr. Gaylord: The difficulty in reproducing x-ray experiments in animals seems, at present, almost insurmountable; yet it should not be allowed to diminish the significance of such positive observations as have been obtained by careful investigators. On the other hand, we should not permit ourselves to draw too sweeping conclusions from their findings.

Dr. Sittenfield has described the induction of lymphocytosis in mice by the use of pilocarpin. A mere increase in the lymphocyte count, however, may not, as such, be significant. In fact, our own observations would strongly indicate that a high lymphocyte count in itself must be regarded more as an indicator of things that have happened in the development of immunity, than as an essential factor in this condition. We have attempted to investigate, in human beings, both the lymphocyte and the polymorphonuclear leucocyte in their relation to x-ray and radium treatment, and have worked out a large number of elaborate charts showing differential counts taken daily or at more frequent intervals. But we have been unable to establish any connection between a successful treatment and the blood count, with the single exception that the introduction of 50 mgm. of radium into the uterine cavity is followed, in the first twenty-four hours, by characteristic fluctuations in the number of polymorphonuclear leucocytes. Perhaps one reason for the greater variations in the lymphocyte count in mice, is the fact that the normal percentage in the mouse is very much higher than in man. Furthermore, it must be remembered that nearly all, if not all, transplanted mouse tumors are accompanied by spirochetes, and that it is yet to be determined whether or not these organisms may not influence the cellular response of the inoculated animal.

Dr. Levin: It is to be expected that Dr. Sittenfield's results should differ from those of Dr. Murphy, inasmuch as the former used hard filtered rays, while Dr. Murphy used very soft ones. The results of the latter investigator are, in fact, really puzzling. The rays employed were so soft as to be absorbed almost entirely by the superficial layer of the derma, yet Dr. Murphy noticed a general effect on the animals. Apparently, much less radiation is required to affect the blood-forming organs than is needed for the skin; moreover, it is possible that the quality of the rays which act on the skin may be different from those which affect the blood-forming organs.

Dr. Loeb: In my opinion, there can be no doubt as to the significance of lymphocytes in tumor and tissue immunity. I first observed lymphocytic infiltration around homoiotransplanted skin in 1909, and since then my collaborators and myself have observed this lymphocytic infiltration around various other tissues after homoiotransplantation. These cells actually destroy the transplanted tissue by invading it and cutting off the blood supply. The connective tissue helps to strangulate the graft after homoiotransplantation, and in certain cases a direct injurious effect is exerted, in addition, by the body fluids. After autotransplantation, this injurious effect of lymphocytes and connective tissue is absent.

The conditions found in tumor immunity are evidently the same as those applying to normal tissues.

12. LIMITS OF VARIABILITY OF TUMOR STRAINS

Dr. Wood:

SUMMARY

The same tumor, when transplanted into a large number of animals, exhibits varying rates of growth; and if the daughter tumors are excised at a stated period it will be found that there are a few large and a few small growths, while the great majority approach an average weight, this average varying with the tumor strain. In experiments undertaken to demonstrate a supposed alteration of the growth rate of a tumor, therefore, it is of the utmost importance that there be employed neoplasms whose biological variations have been determined by observation of large series. For this reason two Crocker Fund mouse tumors, one a carcinoma (No. 11), the other a sarcoma (No. 180), have been carefully observed, and a number of facts concerning their growth rates have been obtained. The standard deviation of the weights of sarcoma 180 in small series of 20 to 200 tumors was found, when the tumors were removed at the end of two weeks after implantation, to be between 15 and 21 mgm., with a mean weight of 162 to 329 mgm. in the different series. The entire series of 1090 tumors had a mean weight of 273 mgm. ± 0.59 mgm., with a standard deviation of 19.84 mgm. ± 0.42 mgm.

With carcinoma 11, the standard deviation was higher, as these tumors, because of a lesser tendency to ulcerate, could be allowed to grow for three weeks and consequently showed more variation in size. Thus the standard deviation in several series of tumors varied from 34.58 to 23.23 mgm., and the mean weight from 318 to 380 mgm. In smaller individual series, very great fluctuations were observed; for instance, in two series of the Ehrlich mouse sarcoma, numbering respectively 227 and 262 tumors, the standard deviation was 20.09 and 30.75 mgm., and the mean weight of the first series was 260 mgm., and of the second 588 mgm. In this last group, there was a considerable number of extremely large tumors, so that the standard deviation was somewhat increased, while the tumors were, on an average, twice as large as those of the preceding series. Such a variation in size is quite as great as that which has been frequently cited as showing the effect, on the growth of tumors, of feeding or of the administration of tumor extracts.

A chart was shown to demonstrate the use of statistical methods in determining the correlation coefficient in a series of 346 tumors. The weight of each tumor and of each animal was found, and a correlation table made up in order to determine whether the size of the animal had anything to do with the size of the tumor; that is, whether large tumors grow in large animals or not. The correlation coefficient being found to be 0.0018, it was evident that there is no such relation. Similar coefficients determined for tumors growing in old and in young animals showed that there is also no relationship between the size of the growth and the age of the host. These observations indicate that the weight and age of the animals, within ordinary limits, are unimportant in experiments of this kind. It is presumed that very aged animals, such as are rarely supplied by dealers, might show less active tumor growth, but this factor is practically negligible because such animals are not used in experimental work.

DISCUSSION

Dr. Levin: The morphological study of animal tumors is undoubtedly an aid to our work, and there is no question that biological statistics offer valuable information. Thus the galtonean mathematical results and the mendelian biological results arrived at the same goal from different starting points. There is one difficulty, however; it is hard to believe that 100 animals from the same source, and fed and housed in the same manner, will not exhibit individual variations due to natural differences in their cellular metabolism. The best evidence that such an event may take place, in the tumor cell at any rate, is the cyclic variations which occur in the growth energy of transplantable neoplasms. Accordingly there appears to be some danger that, while a mathematical calculation worked out to-day may be correct for a time, the whole task will have to be repeated sooner or later, on account of a change in the characteristics of the growth.

Dr. Wood: It has not been asserted that this work is final; it is merely an attempt to remove some of the difficulties connected with work on highly variable material. It is obvious that no one who knows anything of cancer research would employ in experimental work a tumor which regularly disappears in a large percentage of cases, if his aim were to prove the value of some hypothetical therapeutic substance. But if we are ever to obtain any facts on the influencing of the rate of tumor growth, it will be only through smoothing out the fluctuations by such mathematical procedures as I have outlined. It will then be possible to draw conclusions which will stand. These charts show that the use of small series of animals, that is, less than a hundred individuals, may lead to absolutely false conclusions; and the purpose of this work was to determine the normal variations of growth in two standard tumor strains. These two tumors may be employed by other investigators, who will then have at their disposal the statistical results which we have collected, as the Crocker Fund is prepared at any time to furnish tumors of these strains. I realize just as fully as does Dr. Levin that we are working with a highly variable material and that there are many unknown factors in the equation; but if we are to study tumors we must study them growing in animals, and I have attempted to show, solely by statistical methods, that some of the sources of error can be removed by the use of a sufficiently large number of animals.

13. THE REACTION OF BONE TISSUE TO THE GROWTH OF CANCER IN THE WHITE RAT

Dr. Levin and *Dr. Joseph:*

SUMMARY

The Flexner-Jobling rat carcinoma was inoculated into the lower end of the femur in normal white rats. The consequent changes in the bone tissue consisted in a double process: *Osteoporosis*, or destruction of the bone, caused by the direct action of the cancer cells, and *Osteosclerosis*, or new bone formation. The new bone developed from collagen fibrils sprouting from the remnants of the old, the mechanism being similar to that observed by Levin in the development of skeletal metastases in human carcinoma. In another series of animals, the inoculation of tumor into the bone was followed by *x*-ray treatment. Here growth was more scanty, the neoplasm failing to grow in a larger number of animals than in the control experiments. Moreover, the rayed animals exhibited a more extensive formation of collagen fibrils, and consequently of new bone.

14. A REPORT OF THE ANALYSIS OF THE BLOOD IN CANCER PATIENTS FOR THE NON-PROTEIN CONSTITUENTS

Miss Ruth Theis and *Dr. Stanley R. Benedict* (New York):

SUMMARY

The work has included the determination of total non-protein nitrogen, urea, uric acid, and sugar, in about one hundred patients suffering from malignant tumors of various types. In addition to the above constituents, amino-acid nitrogen was determined in a considerable percentage of the cases. The results indicate that the total non-protein nitrogen of the blood in cancer patients is slightly below normal. The figures for urea nitrogen also average below the normal, both relatively and absolutely. The amino-acid nitrogen is slightly higher than the normal figures. Uric acid is normal in amount, except in one group of three melanoma cases, where this substance is distinctly above the normal. In this connection, it is of interest to note that melanoma contains a substance giving a marked reaction with the Folin-Denis uric acid reagent, but which is certainly not free uric acid. The nature of this substance is being investigated.

DISCUSSION

Dr. Wells: Is the high figure obtained in the blood in melanoma cases surely due to uric acid, or merely to material reacting with phosphotungstic acid? Tissues will give figures by Folin's method entirely unexplained by the results obtained through direct determination of uric acid, and Dr. Folin has told me that he has had the same experience with tissue extracts. Folin's method cannot be used for tissue uric acid. The slight increase of uric acid that is found in the blood might possibly be ascribed to the fact that there is frequently a considerable degree of injury to the kidneys in advanced age.

Dr. Benedict: In regard to the question about uric acid, I may say there is little question that the substance is 95 per cent uric acid. In ox blood I found 50 per cent more uric acid than Folin did. If one works carefully with ox blood, one can isolate more than 90 per cent of the uric acid found colorimetrically. The color reaction gives a precipitate with ammonia, silver, and magnesium. With melanoma extract, the color reaction is not obtained with the precipitate. In regard to kidney involvement, the figures for urea and total nitrogen are inclined to run up where there is impairment.

Dr. Wells: Urea is retained when uric acid is not.

Dr. Benedict: The total non-protein nitrogen is below normal in our cases. Apparently urea is excreted even better than normally in our cases, so that the chance of impairment of the kidney is not great.

15. Growing Spleen Exerts no Influence upon the Proliferation of Tumors in the Chick Embryo

Dr. H. N. Stevenson (New York):

SUMMARY

The experiments to be reported were undertaken to determine the influence of spleen upon tumors of the rat and mouse growing in the chick embryo, an investigation which seemed to be necessary because the inhibitory action of spleen reported by Murphy was not observed by Bullock in an analogous growth medium. Bullock found that newborn rats have a period of susceptibility similar to that of the chick embryo, but he was unable to terminate, with adult rat spleen, this period of susceptibility to mouse tumors.

The technique employed in the investigation described here was similar to that used by Murphy. The eggs were inoculated on the seventh day of incubation with tumor and bits of adult chicken spleen, and the grafts removed on the seventeenth or eighteenth day of incubation. The dosage employed was 0.003 gram[1] of tumor and 0.005 gram of adult chicken spleen.

Six tumors were used; the Imperial Cancer Research Fund mouse carcinoma 63; the Crocker Fund mouse carcinoma 11; the Crocker Fund rat sarcoma 8; the Jensen rat sarcoma; the Ehrlich mouse sarcoma; and the Crocker Fund mouse sarcoma 180. Immunity can be induced in the animal against the first four, but can not be induced in the animal against the last two.

It was found that all the tumors used grow in the presence of adult chicken spleen without any sign of inhibition. The reaction on the part of the wandering-cells is increased when growing spleen is present, as compared with that seen in control grafts. This reaction is made up almost entirely, however, not of lymphocytes, but of cells belonging to the myeloid or granular leucocyte series.

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[1] In previous publications from the Imperial Cancer Research Fund and from this laboratory, the inoculation dose, when the needle method is used, has been estimated as 0.01 or 0.02 gram; but such grafts have recently been found, as a matter of fact, to weigh about 0.002 and 0.003 gram respectively.

Lantern slides of the six tumors were shown, each having a graft of growing spleen in the same section. These illustrated the numerous mitotic figures in the tumors, the increased wandering-cell reaction, and the healthy condition of the splenic grafts.

DISCUSSION

Dr. Murphy: I have heard this paper once before. The chief criticism I have to make refers to the size of the spleen grafts, which is smaller than that used in my experiments. The results are interesting, but must be carefully controlled. With the Jensen rat tumor, we obtained approximately 100 per cent takes in the embryo. We saw not a single failure of inhibition, when the spleen graft had taken satisfactorily; we say inhibition because the tumor cells were not invariably killed. The microscopic picture is similar to the first slide shown by Dr. Stevenson. A few mitotic figures, I think, have no significance, for they are often seen in numbers in a failing heteroplastic graft. If the Jensen sarcoma happens to be more easily influenced than some other tumors, it does not alter the fact that spleen is capable of exerting a distinct influence. Besides the size of the spleen graft, another point of difference between Dr. Stevenson's work and mine is, that he used solid bits of tumor and spleen, while I used both materials, hashed and injected with a syringe.

Dr. Woglom: Does Dr. Murphy regard the immunity conferred by chicken spleen against a rat tumor growing on chicken soil, as similar to that conferred by rat tissues against transplanted rat tumors growing on rat soil? The cases do not appear to be similar, for in the latter the tumor is growing amid homologous surroundings, while in the egg it is in a heterologous medium. Hence it would appear to be hazardous to argue from one to the other.

Dr. Murphy: I cannot answer that question; there is no way of deciding the point. The immunity of chick embryos to the tumor is at any rate, the same as that found in the adult fowl. Foreign tumors will grow up to the nineteenth day of inculation, but retrogress in the following twenty-four to forty-eight hours; tumors which measured 1 cm. have completely disappeared within twenty-four hours after the chick had hatched. Our impression was, that the spleen grafts brought about this same type of reaction at an earlier period, rather than the condition displayed by mice immunized against cancer inoculation; for the reaction in the embryo seems to depend on the survival of the spleen graft.

Dr. C. R. Stockard (New York): If these experiments of Dr. Stevenson are to be considered from the standpoint of a competition between transplanted tissues, it becomes difficult to compare them with those of Dr. Murphy. We must first take into account the relative growth vigor of tumor and spleen after implantation in the yolk membranes of the chick embryo, which has not been done. In any individual experiment, the piece of tissue which becomes attached and grows first has the advantage. The method and site of the implantation are very important, and of even greater significance is the relative size of the two pieces of tissue used. As one looks at the lantern slides, this work and Dr. Murphy's appear to be very different; in one case definite grafts of spleen and tumor were implanted, while in Dr. Murphy's experiments an emulsion was injected. There are thus so many differences that it seems impossible to make any comparison of value between these experiments and those of Dr. Murphy.

Dr. Wood: It seems to me that the question under discussion is neither the details of the inoculation site, nor the methods of implantation, nor the quantity of spleen used. Dr. Murphy has asserted that spleen and tumor will not grow together in the chick embryo; Dr. Stevenson merely shows that spleen and tumor will grow together in the chick embryo. One positive result of this sort is worth more than a thousand negatives.

Dr. Stockard: But how long do they grow together? If the chicks were not killed, would the spleen finally get ahead of the tumor? These questions have not been answered by the experiments. If two pieces of equal size, spleen and tumor, are planted, how many days does it take the spleen to overcome the tumor? In any competition between growing tissues we must consider the amount of one tissue necessary to overcome the other in any given length of time. It can hardly be imagined that spleen could suppress a proliferating tumor immediately, even though in time it might have the power eventually to overcome it. Comparable phenomena are supplied by normal embryological development, in which all retrogressive tissues and organs are overcome as development advances. Here either the appearance of some other structure, or the action of the organism as a whole, causes the degeneration of the given tissue; yet in the case of the human organism some of the retrogressive structures may maintain themselves even for years in spite of the competition. Thus both quantitative and time elements must be considered, if experiments on tissue competition are to be exact.

Dr. Gaylord: Mitotic figures do not in themselves mean progressive growth of a tumor. They are to be found in cells representing the last remnants of retrograding mouse tumors.

Dr. Murphy: I am sorry that Dr. Stevenson has not shown charts of the sizes of the control tumors. These, it seems to me, are of more importance than mitotic figures in judging the rate of growth.

Dr. Gaylord: Did you mix the spleen and tumors together in your experiment?

Dr. Murphy: No. They were injected on different sides of the eggs. And in one series, the spleen grafts were introduced two days later than the tumor grafts.

Dr. Gaylord: I thought, from what Dr. Stevenson said, that he mixed them together.

16. Tissue-Stimulating Effects of Placental Extracts

Dr. Robert T. Frank (New York):

SUMMARY

Various investigators, notably Iscovesco, Fellner, Aschner, and Herrmann, have described the stimulating effect produced on the uterus by the lipoid fraction of placental and corpus luteum extracts. The present investigation was planned to analyze these effects.

Standardization of the extract is effected by giving immature rabbits four injections in eight days, and then comparing the length, thickness, and weight of the uterus with a control. An increase in size, arbitrarily designated as from zero to plus three, can be obtained. The increase obtainable requires proportionately higher dosage as the upper limit is approached.

The uterus shows marked hyperplasia of all its layers, with the exception of the peritoneal coat. The epithelium increases in height, in the number of its layers, and in the size of its individual cells. The connective tissue, likewise, shows multiplication and an increased succulence. Both epithelium and connective tissue contain many mitoses. The musculature becomes thicker, but no mitoses are found. The water content of the stimulated uterus is higher than that of the control.

These uterine changes occur independently of the ovaries, which are not stimulated, and in castrates after the ovaries have been removed. They also occur in animals deprived of their thyroid, adrenal, or pancreas, or of their thyroid and adrenal. Atrophic uteri are still susceptible to the stimulus as long as sixteen months after castration.

The extracts stimulate similarly the breast, producing a great increase in its area and in the development of its ducts, acini, and nipples.

The uterine reaction can be obtained in rabbits, guinea-pigs, cats, and rats. The breast reaction is less distinct in guinea-pigs and rats.

Hyperplasia occurs in pieces of uterus transplanted subcutaneously or intramuscularly. Upon stimulation, the transplants show greater vitality than do the controls. This applies especially to pieces of uteri transplanted into other animals of the same species. Previously stimulated uteri transplanted into other animals, and again stimulated, show no difference from ordinary transplants which are stimulated.

Upon injecting small quantities of all the layers of the uterus in a finely subdivided state, either subcutaneously or intramuscularly, and then stimulating, small masses develop which increase in size during a period of from two to four weeks, and then slowly regress.

These artificial tumors resemble adenomyomata of the uterus, each epithelial group forming a tube and being surrounded by a connective tissue layer (submucosa) and a layer of musculature. Only in one instance was an isolated (probably regressing) group of solid epithelial cells encountered.

This investigation proves:

1. That there are isolable growth substances in the animal organism which exert a stimulating effect upon two groups of organs—one derived from Müller's ducts, and the other from the skin and its appendages.
2. That in both groups the epithelial as well as the connective tissue and muscle cells respond to the stimulus.
3. That transplantation does not interfere with the growth reaction, and that transplanted stimulated tissues show greater vitality in their new environment than unstimulated controls.
4. That the growth impulse is effective after advanced and prolonged atrophy has supervened.
5. That it has proved impossible to stimulate growth beyond normal (i.e. pregnancy) limits, and that finely divided cell groups show a strong tendency to reconstruct along normal groupings and configurations.

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